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MEDICAL RESEARCH COUNCIL

Borna Disease and Enzootic Encephalo-Myelitis of Sheep and Cattle

BY

S. NICOLAU, M.D., D.Sc., and
I. A. GALLOWAY, B.Sc., M.R.C.V.S.

LONDON
HIS MAJESTY'S STATIONERY OFFICE
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PREFACE

THE present report, which was received for publication in October, 1927, gives the results of investigations made by Dr. Nicolau and Mr. Galloway, working as guests in the National Institute of Medical Research. These studies of the virus which is responsible for a dangerous communicable disease in horses, cattle and sheep have happily no immediate practical application in this country. Borna disease and its congeners are at present only known in continental Europe and in America. Our present immunity, however, may only be temporary, and in any case it is highly important that we should have the fullest knowledge of these epizootic diseases of other countries.

The scientific advantages gained by close association between studies of disease in animals and studies of human disease have long been obvious. The potential value to medical science of the work now presented lies in two directions. The accurate experimental study of the ' virus ' which is the causal agent in this disease is part of the general study of disease viruses, and in this field of work great gain must come from the free exchange of ideas, methods and results among workers in different parts of it. Besides this, however, Borna disease has special points of interest to students of human neurology. The infective virus produces changes in the brain and spinal cord, the so-called encephalo-myelitis, which throw light upon analogous forms of encephalitis and myelitis which occur in sporadic or epidemic form in human beings.

It will be seen that the authors have extended or confirmed the observations of many previous workers, and have gained new knowledge at several points of detail by their experimental work. Fresh studies have been made of the immunity reactions of the virus, and it has been shown that animals may be successfully immunized against it.

Dr. Nicolau was enabled to conduct this work in the National Institute by a grant from the Roumanian Government. Mr. Galloway co-operated with him in the course of other work upon foot and mouth disease in cattle, which he is doing on behalf of the Ministry of Agriculture and Fisheries. The council are indebted to the Ministry for the facilities they have given for this co-operation.

28th July, 1928.

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BORNA DISEASE AND ENZOOTIC ENCEPHALO-MYELITIS OF SHEEP AND CATTLE

By S. Nicolau, M.D., D.Sc., and I. A. Galloway, B.Sc., M.R.C.V.S.

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CONTENTS

1. HISTORICAL AND GENERAL

I. Enzootic Encephalo-myelitis of the Horse (Borna Disease).

The disease has various designations: Enzootic Encephalomyelitis, Meningo-encephalo-myelitis of the Horse, Névraxite enzootique; Mal d'Azeau (after the region where a severe epizootic occurred in Belgium in 1909); Bornasche Krankheit, Genickstarre, Gehirnruckenmarksentzündung, Gehirnruckenmarkseuche, Nervenfieber; Encephalitis Lymphocytaria Equi.

It is an infectious malady with a mortality rate reaching 90 per cent. in some epizootics. The characteristic symptoms are due to lesions in the nervous system both central and peripheral, which are produced by an ultravisible and filterable virus.

The name Borna disease, by which the disease is more commonly known, originated from the locality in Saxony where a severe epizootic occurred in the years 1894 to 1896.

Borna disease has been known for more than a century. It was first described by Wörz in Würtemburg in 1813. Subsequently it was reported from Germany, North America, the Argentine, and Hungary. Since 1900 it has appeared in Russia, in the region of the river Don, in Belgium, France, Italy, Roumania, Germany, and South America. The epizootics have been of varying severity. In 1896 the disease occurred in several districts of Germany, where 1,198 horses were reported to have contracted it; in the epizootic which occurred in the valley of the Colorado and the Brazos in America about 4,000 horses and mules succumbed. In other areas the disease has appeared sporadically.

Climatic conditions appear to have an influence on the spread of the disease, the incidence of which is greater when abnormally warm and moist conditions obtain. The diagram published by Zwick, Seifried, and Witte (1926) shows that epizootics generally begin in the month of May and decline later, the cases again becoming sporadic during the winter months.

The period of incubation in the spontaneously contracted disease is difficult to estimate and is therefore not definitely known. Noack (1908) considers 9 days to be an average.

Joest (1926) suggests that the portal of entry of the virus is through the nose, but the possibility of ingestion being the mode of infection cannot be excluded.

The onset of the disease may be sudden, but some authors have reported that fatigue, gastro-intestinal disturbances, and symptoms of affection of the upper respiratory passages precede the onset of characteristic nervous symptoms by as much as 14 days.

The first symptom which usually draws attention to the infected animal is lassitude: the horse is easily fatigued and appears depressed and indifferent to external impressions. A period of excitation follows, which may last with intermittence till the end of the disease. Tonic

contractions of diverse groups of muscles occur and there is difficulty in mastication and deglutition. During the stage of excitation external stimuli produce exacerbation of the spasms in certain groups of muscles; champing of the jaws is a common symptom, and saliva flows from the commissures of the mouth. The pupils are unequal in size. Soon paresis or paralysis sets in, affecting the hindquarters, the muscles of the tail, muscles of mastication, muscles of the tongue and of the back to a varying degree. Paraplegia or hemiplegia are not uncommon.

In some cases the symptoms of encephalitis are dominant, in some those of acute myelitis are more evident, while in others symptoms characteristic of affection of both brain and cord coexist. The temperature generally remains normal throughout, and according to Hutyra and Marek (1922) persistent fever indicates secondary complications of a septic nature.

The frequency of the respiration remains little changed, except during periods of excitation, or when the nucleus of origin of the vagus nerve is involved. Towards the end of the disease, however, the respirations become superficial and approach the Cheyne-Stokes type.

The examination of the blood and urine does not indicate any constant change. The cerebrospinal fluid shows lymphocytosis occasionally. The duration of the disease varies from a few days to 6 weeks.[1]

Aetiology. As has been the case with a number of diseases subsequently proved to be due to a filterable virus, the disease was at first attributed to the pathogenic action of various cocci. Siedamgrotzky and Schlegel (1896) isolated a Gram-positive diplococcus and Johne (1896) a Gram-negative one. Other observers, Ostertag (1900), Christiani (1909), Marcq (1909), Löhr (1910), Lessage and Frisson (1912), have found streptococci or diplococci, each organism isolated being considered by the investigator concerned as the aetiological agent. More recently Kraus, Kantor, Fischer, and Quiroga (1920) isolated a diplococcus similar to that found by Siedamgrotzky and Schlegel, which they believed to be the aetiological agent of Borna disease until the appearance of the work of Moussu and Marchand in France, and Zwick, Seifried, and Witte in Germany, indicating that encephalo-myelitis of the horse is produced by an ultravisible and filterable virus. Although the virus isolated by Moussu and Marchand appears to differ from that isolated by Zwick and his collaborators, it is possible that this may be explained by the fact that two strains of the same virus were being dealt with (see note on p. 23). Beck and Frohböse (1926) and Ernst and Hahn (1926) also showed that a virus isolated from the brain of horses affected with encephalo-myelitis was capable of infecting rabbits.

Joest in collaboration with Degen (1909) determined the constant

[1] In the epizootics described by R. Moussu (1926) the duration of the disease was very short. According to this author, in those cases showing symptoms of an encephalitic type 'the evolution is rapid. In the cases we have studied death intervened in 29, 32, and 37 hours.'

presence of certain intranuclear 'inclusions' in the ganglion cells of the Ammon's horn which they considered as reactions of the cell to the parasite and 'similar to Chlamydozoa'. In the opinion of Joest these chlamydozoa, or at least certain forms of their cycle of development, are capable of passing through bacteriological filters.

Siedamgrotzky and Schlegel (1896), Lohr (1910), and Ostertag (1900 et seq.) made frequent attempts to infect laboratory animals by various methods of inoculation or by feeding them with cerebrospinal fluid, blood, emulsions of spleen, liver, kidney, or bone marrow from horses affected with the disease. The results were negative.

In *résumé* the work of Moussu and Marchand (1924 et seq.), Zwick, Seifried, and Witte (1926), Beck and Frohböse (1926), Ernst and Hahn (1926–7), has shown definitely that the aetiological factor is a filterable ultravisible virus, and the results obtained by Kraus and his collaborators (1920) and other investigators with their cultures of diplococci or diplo-streptococci must now be considered as due to what Nicolle called *microbes de sortie*.

II. Enzootic Encephalo-myelitis of Cattle.

The epizootiology and symptoms of encephalo-myelitis of cattle are similar to Borna disease of the horse. Hutyra and Marek (1922) refer to outbreaks of disease amongst cattle described by Meyer (1867), Schmidt (1888), Utz (1896), Röder (1896), and Manfredi d'Ercole (1896) which may be attributed to enzootic encephalo-myelitis. Pröger (1896) recorded the coexistence of this disease with Borna disease, and as Ernst and Hahn (1927) have suggested that the outbreaks of disease in Hungary referred to as 'cerebrospinal meningitis' may also have been encephalo-myelitis. G. Moussu, quoted by R. Moussu (1926), in 1906 studied an enzootic on a farm in the region of Orne which killed 10 cattle in a few weeks. The disease had a similar onset; periods of excitation were observed followed by depression with champing of the jaws, salivation, loss of vision, and muscular twitchings. The possibility of intoxication was excluded, and the symptoms were suggestive of Borna disease. Moussu also considers that the cases of 'cerebrospinal meningitis' of cattle reported by Kragerud and Gunderson (1921) were the same as encephalomyelitis of the horse, and the symptoms described by Causel (1924) in an enzootic of what he termed 'infectious bulbar paralysis' amongst cattle are consistent with the view that the animals suffered from encephalo-myelitis.

The symptoms of encephalo-myelitis in cattle are similar to those of Borna disease in the horse.

Aetiology. Moussu (1926), who described 31 cases of encephalomyelitis of cattle on 18 different farms in France, attempted to transmit the disease to laboratory animals without success.

Ernst and Hahn (1927) found lesions in the brain of cattle dead of encephalo-myelitis analogous to those in the brain of horses dead of Borna disease; in the lesions the corpuscles of Joest-Degen were seen. With the virus which they recovered they succeeded in producing

symptoms in rabbits similar to those produced by Zwick with a virus from horses.

The general aspect of the lesions in the brain of rabbits infected with the two viruses and the similar presence of the corpuscles of Joest-Degen suggests that varieties of the same virus are the cause of encephalo-myelitis of horses and cattle.

III. Enzootic Encephalo-myelitis of Sheep.

Eichbaum, Stöhr, and Wilke (1865, 1866) recorded an enzootic of encephalo-myelitis among sheep, and Roloff (1868), who examined the brain of animals which succumbed to a similar epizootic, found perivascular infiltration in the pia mater. Schmidt (1870), about the same time, described an enzootic of a similar nature in Prussia. Later, Popow (1882) and Wischnikowitsch (1889) described the disease in Russia. Prietsch (1896) referred to an enzootic among sheep, and suggested the possibility that the source of infection was the water in troughs contaminated by the virus of Borna disease of horses. Walther (1899) reported an enzootic in two flocks of sheep in the district of Borna at a time when equine encephalo-myelitis was prevalent; the two diseases, equine and ovine, bore many resemblances to one another. Savigné and Leblanc (1897) also have described an enzootic in France.

The descriptions of these authors differ essentially and may not all refer to the same disease. More precise accounts of ovine encephalo-myelitis have been published within recent years by Spiegl (1922), Priemer (1925), Beck and Frohböse (1926), Moussu (1926), Miessner (1926), and others, from which it appears that in the spontaneous disease of the sheep the same succession of symptoms occur as in horses and cattle. The evolution of the disease may take as long as from 2 to 12 days. Death generally supervenes. The temperature is variable. In certain cases the temperature may rise to 41° C., while in others no pyrexia is observed. According to Moussu (1926) the incubation period averages 27 days.

Aetiology. Beck (1925) studied the microscopic lesions in the brain of sheep dead of the disease, and emphasized their resemblance to those found in the brain of horses dead of Borna disease. In the ganglion cells of the Ammon's horn of sheep the characteristic oxyphilic intranuclear corpuscles of Joest and Degen were found. In collaboration with Frohböse, Beck (1926) transmitted the disease to rabbits, and from the similarity in the symptoms and in the lesions produced by the virus isolated by them from sheep with that isolated by Zwick from horses, considered that the two diseases were identical.

About the same time Moussu and Marchand also passed the disease to rabbits and transmitted it from sheep to sheep. Later Miessner (1926) and Ernst and Hahn (1927) confirmed the transmissibility of the disease of sheep to rabbits.

We shall describe later how the experimental study of enzootic encephalo-myelitis of sheep has shown that the disease is produced by a virus similar, if not identical, with that which produces Borna disease in horses.

IV. Malignant Catarrhal Fever of Cattle.

Glamser (1926) and Dobberstein (1925) found perivascular and parenchymatous infiltration as well as alterations in the ganglion cells of the brain of cattle dead of a disease which they called malignant catarrhal fever. These lesions were similar to those described in Borna disease. Ernst and Hahn (1927) also draw attention to the similarity of the lesions in the brain of cattle dead of this disease with those found in encephalo-myelitis of the horse, and in 3 out of 5 cases they observed the intranuclear corpuscles of Joest-Degen in the ganglion cells of the Ammon's horn. Emulsions from the brain of one of these cases inoculated intracerebrally into rabbits produced a disease similar to experimental Borna disease and transmissible from rabbit to rabbit. They concluded that malignant catarrhal fever of cattle is produced by a virus which approaches very closely, if it be not identical with, that producing encephalo-myelitis in horses.

We might here mention that Ernst and Hahn (1927) also made an observation indicating that deer are susceptible to Borna disease. A sick deer was killed by a hunter under curious circumstances. The animal allowed the hunter to approach very closely, drew away in fear, and then rushed on him suddenly. The ears were seen to twitch and the animal turned round in a circle until the hunter, probably more frightened than the animal, killed it. The head was brought to Munich. The brain showed the presence of lesions similar to those of Borna disease of the horse, and the intranuclear corpuscles of Joest-Degen were observed. Their attempts at transmission of the disease to laboratory animals had given no results at the time of publication of these observations.

V. Summary.

From the foregoing *résumé* of the literature of spontaneous encephalo-myelitis in different species, the following conclusions may be drawn:

(1) The enzootic encephalo-myelitis of horses and cattle and of sheep appears to be the same disease. The symptomatology and the lesions found in the central nervous system are analogous, and the intranuclear corpuscles of Joest-Degen occur in the large ganglion cells of the Ammon's horn in all three species suffering from the disease in question.

(2) From cases of all three diseases a virus has been recovered and shown to be responsible for the disease.

(3) From the observations of Ernst and Hahn it would seem not improbable that, if the animals had not in addition to malignant catarrhal fever a concomitant infection with Borna disease, some of the cases described as malignant catarrhal fever of cattle were encephalo-myelitis.

(4) Deer appear to suffer from a similar disease spontaneously.

(5) The transmission of the disease under natural conditions is probably by the respiratory tract or by ingestion.

2. PROPERTIES OF THE VIRUS

I. Invisibility.

The virus in ultravisible. Various methods of staining have been used to discover a parasite, but without success. Methods of impregnation with silver have not revealed the presence of parasites in the brain of animals dead of the experimental disease in our hands. The existence of the virus in the brain is, however, associated with the presence of intranuclear corpuscles, first described by Joest and Degen (1909). The interpretation of the nature of these corpuscles which we favour is discussed in the chapter dealing with the histopathology of the disease, and we are persuaded that they are of the same nature as the similar 'inclusions' found in other diseases produced by filterable viruses, such as fowl plague, fowl pox, distemper, and 'Virus III' disease of rabbits.

II. Filterability: Effect of Dilution.

Filtration of emulsions containing viruses through filters which hold back bacteria generally greatly diminishes the concentration of the virus. There are many reasons for this, apart from the size of the virus. If the virus is contained in or on cellular particles these may be retained, or the virus itself may be adsorbed on the filter. In such adsorption the hydrogen-ion concentration and the electrical charge carried by the virus exert a decisive influence. The pressure under which the filtration is carried out is also a factor of importance.

Further, the sensitiveness of the tissue into which a filtrate is inoculated may influence the decision whether a virus is filterable or not. When a sensitive reagent is employed, the passage of an extremely small quantity may be detected. For instance, the dose of neurovaccinia required to infect a rabbit if inoculated intracerebrally is 1/100th to 1/1000th of that required to produce pustules on application to the scarified skin. An experimenter using the former method might conclude that the virus passed a bacteria-proof filter, one using the latter that it did not. The sensitiveness of the tissue into which a filtrate is inoculated is therefore a factor of capital importance often neglected in interpretations as to the filterability or non-filterability of the virus.

The experiments of Moussu (1926), Zwick, Seifried, and Witte (1926), and Ernst and Hahn (1927) show that the virus of Borna disease can pass through ordinary bacteriological filters, but that filtration of the virus is not easily effected. Zwick carried out more than thirty filtration experiments with different filters and inoculated more than 100 rabbits with the various filtrates before he succeeded in infecting the animals with a filtrate.

We have carried out two filtration experiments, using the following technique: from the brain of Rabbit 275, which died on the 32nd day after cerebral inoculation and exhibited characteristic lesions throughout the nervous system, an emulsion 1 : 30 was made with

physiological saline. The emulsion was placed in the ice-chest for three hours to allow the coarser particles to deposit. The supernatant fluid was filtered by aspiration under low pressure through a Mandler filter.

Experiment 1.

The filtrate was inoculated intracerebrally into Rabbits 218A, 168A, 228A, and 201A, on the 15.3.27.

During a period of 174 days following the inoculation Rabbit 218A did not show any symptoms, and its weight increased in an even curve by 730 gms. After this period had elapsed the resistance of the animal was tested with fresh passage virus by intracerebral inoculation. It died in 37 days after showing typical symptoms of the disease. On microscopic examination of sections of the central nervous system characteristic lesions were found.

Rabbit 168A, weighing 700 gms., and *Rabbit 228A*, weighing 2,080 gms., also remained well for 174 days following the inoculation, and their weights increased to 1,330 gms. and 2,640 gms. respectively. They were both reinoculated intracerebrally with fresh passage virus on September 5, 1927 (174th day since inoculation), and proved not to be refractory to infection; Rabbit 168A dying 14 days and Rabbit 228A 37 days after inoculation with the test dose. The control rabbit, 25A, inoculated intracerebrally on the same date as the others, died 48 days after infection, and typical lesions were found in the central nervous system,

Rabbit 201A was inoculated intracerebrally with filtrate on the 15.3.27.

Observations.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,500	
20.3.27	1,620	Normal.
25.3.27	1,660	Normal.
31.3.27	1,520	Animal appeared to be ill. Rabbit found
2.4.27		dead 18 days after inoculation.

On the autopsy no abnormal condition of the organs was observed and the cultures of the brain remained sterile. On microscopic examination slight infiltration and perivascular lesions were found in the brain, mid-brain, and spinal cord, and, in addition, a marked pathological 'satellitism' of the nerve-cells. Passage was made with the brain of this rabbit to a healthy rabbit, No. 260A, with the results given in detail below:

Rabbit 260A, weighing 1,740 gms., was inoculated intracerebrally with an emulsion of the brain of *Rabbit 201A*.

It developed a disease of a *recurrent* nature and succumbed after the second crisis, 161 days after inoculation.

Autopsy. All organs macroscopically normal.

Cultures in broth from the brain remained sterile.

Microscopic examination. Intense and characteristic lesions were found in the brain and in the cord. The intranuclear corpuscles of Joest-Degen were also found.

Observations.

Date.	*Weight in gms.*	*Clinical observations.*
2.4.27	1,700	Nothing abnormal in animal's condition.
19.4.27	1,800	,, ,, ,, ,,
28.4.27	1,900	,, ,, ,, ,,
5.5.27	1,920	,, ,, ,, ,,
12.5.27	1,600	Depressed, posterior paresis.
16.5.27	1,570	Placed on his flank the animal made several vain efforts to recover its normal position.
21.5.27	1,620	Condition ameliorated—slight paresis of hindquarters.
30.5.27	1,600	Much improved.
13.6.27	1,640	Normal.
22.6.27	1,740	,,
30.6.27	1,770	,,
15.7.27	1,800	,,
1.8.27	1,700	,,
8.8.27	1,540	Animal ill.
15.8.27	1,300	Paresis of hindquarters.
24.8.27	1,180	,, ,,
31.8.27	1,000	,, ,,
9.9.27	980	Head depressed, paresis of hindquarters, typical symptoms of the disease.
12.9.27	820	Placed on the flank, the rabbit made vain efforts at recovering the normal position.
13.9.27		Found dead 161 days after inoculation.

Experiment 2.

A second filtration experiment was carried out with the same technique. The filtrate through a Mandler filter was inoculated into the brain of four rabbits, 150A, 152A, 210A, and 231A.

*Rabbit 231*A died accidentally 5 days after inoculation.

*Rabbit 210*A remained unaffected and gained progressively in weight.

The protocols of Rabbits 150A and 152A are given below.

*Rabbit 150*A.			*Rabbit 152*A.	
Date.	*Weight in gms.*	*Clinical observations.*	*Weight in gms.*	*Clinical observations.*
15.3.27	1,800		2,200	
20.3.27	1,880		2,440	Normal.
31.3.27	1,860		2,440	,,
15.4.27	2,260		2,500	,,
28.4.27	2,320		2,660	,,
5.5.27	2,520		2,680	,,
12.5.27	2,420	Slight paresis behind.	2,620	,,
21.5.27	2,340	,, ,,	2,680	,,
30.5.27	2,340	Condition improved.	2,620	,,
5.6.27	2,460	Normal.	2,650	,,
13.6.27	2,640	,,	2,790	,,
29.6.27	2,700	,,	2,700	,,
15.7.27	2,900	,,	2,650	,,
1.8.27	2,970	,,	2,600	,,
29.8.27	3,000	,,	2,650	,,

Therefore only Rabbit 150A showed slight transitory symptoms which ultimately passed away completely. These symptoms may have been due to the inoculation of a small dose of virus.

In order to determine whether the 3 rabbits, 150A, 152A, and 210A, had developed any degree of immunity as a result of inoculation with the filtrate, 167 days after the first injection they received intracerebrally a virulent emulsion of brain at the same time as a normal rabbit, 25A, which served as a control. Rabbit 150A died on the 49th day, Rabbit 152A on the 40th day, and Rabbit 210A on the 40th day, and the control rabbit on the 48th day after inoculation of the test dose. Typical lesions were found in all cases on microscopic examination of sections of brain and spinal cord.

Conclusions. The results in our few experiments support the conclusion of Zwick, Seifried, and Witte (1927) that the virus of Borna disease can pass, though with great difficulty, through bacteriological filters which retain ordinary bacteria, and that the concentration of virus in the filtrate is much reduced.

Zwick succeeded in obtaining an active filtrate after filtering a virulent emulsion of brain through a Zsigmondy Bachmann collodion membrane, of which the size of the pore was estimated to be 0·75 μ.

Effect of dilution. Few titration experiments have been made. Zwick records that a virulent emulsion of brain is still capable of producing the disease in a dilution of 1 : 10,000.

III. Centrifugalization.

Experiment 1.

A homogeneous emulsion of virulent brain was made, and after the larger particles had been allowed to deposit, the supernatant fluid was pipetted and centrifugalized for 5 minutes at 5,400 revolutions per minute. The supernatant fluid after centrifugalization was carefully pipetted off and inoculated intracerebrally into three rabbits weighing between 1,300 and 1,500 gms. These three rabbits fell ill, showed typical symptoms and died, 39, 48, and 90 days respectively after the inoculation. The characteristic lesions of Borna disease were found in the central nervous system of all three.

(*a*) Rabbit 322B. Weight 1,540 gms.
(*b*) Rabbit 321B. Weight 1,300 gms.
(*c*) Rabbit 320B. Weight 1,500 gms.

The inoculation was made on 15.3.27. The protocol of the three rabbits is given below:

Rabbit 322B.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,540	
23.3.27	1,680	Normal.
25.3.27	1,700	,,
31.3.27	1,700	,,
10.4.27	1,620	,,
15.4.27	1,580	,,
19.4.27	1,420	Paresis of hind quarters.
28.4.27	1,220	Typical symptoms of the disease.
2.5.27	—	Found dead 48 days after inoculation.

Culture of the brain. Negative.
Microscopic examination. Intense and characteristic lesions in the central nervous system.

Rabbit 321B.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,300	
23.3.27	1,280	Normal.
25.5.27	1,380	,,
31.3.27	1,420	,,
10.4.27	1,480	,,
15.4.27	1,600	,,
19.4.27	1,640	,,
28.4.27	2,000	,,
10.5.27	2,100	,,
21.5.27	2,320	,,
30.5.27	2,080	Paresis of hindquarters.
5.6.27	1,800	Typical symptoms of disease.
11.6.27	1,580	Coma. Killed 90 days after infection.

Cultures of the brain. Negative.

Microscopic examination. Intense and characteristic lesions in the central nervous system.

Rabbit 320B.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,500	
23.3.27	1,600	Normal.
25.3.27	1,620	,,
31.3.27	1,440	,,
10.4.27	1,320	,,
15.4.27	1,320	Paresis ?
19.4.27	1,140	Typical symptoms of the disease.
23.4.27	1,020	Died 39 days after infection.

Cultures from the brain. Negative.

Microscopic examination. Mild, but characteristic lesions in the central nervous system.

Experiment 2.

A virulent emulsion of brain was allowed to deposit, and the supernatant fluid centrifugalized as in the last experiment (5,400 revs. per minute) for 15 minutes. Two rabbits were inoculated intracerebrally with the supernatant fluid. Both these rabbits developed the disease typically with paralysis, and died 28 and 45 days respectively after the inoculation, showing lesions of a characteristic nature in the central nervous system.

(a) *Rabbit 324B.* Weight 1,150 gms.

(b) *Rabbit 326B.* Weight 1,150 gms.

The inoculation was made on 15.3.27.

The protocol of these two rabbits is recorded below.

Rabbit 324B.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,150	
20.3.27	1,360	Normal.
25.3.27	1,400	,,
31.3.27	1,330	,,
11.4.27	1,140	,,
15.4.27	1,160	,,
19.4.27	1,160	Commencement of symptoms.
28.4.27	1,080	Paralysis of hindquarters.
29.4.27	—	Found dead 45 days after inoculation.

Cultures from the brain. Negative.

Microscopic examination. The brain and spinal cord showed the presence of typical lesions.

Rabbit 326B.

Date.	*Weight in gms.*	*Clinical observations.*
15.3.27	1,150	
20.3.27	1,360	
25.3.27	1,440	
31.3.27	1,380	
11.4.27	1,020	Paresis of hindquarters.
12.4.27	—	Found dead 28 days after inoculation.

Microscopic examination. The brain and spinal cord showed the presence of typical lesions.

Conclusion. Centrifugalization for even 15 minutes at 5,400 rev. per minute does not deprive the supernatant fluid of virulence. This fact, in addition to the properties of filterability and invisibility of the pathogenic agent suggests that the size of the infective element is excessively small. It is affected by centrifugalization in the same way as other filterable viruses such as those of foot-and-mouth disease, rabies, herpes, vaccinia, and poliomyelitis.

IV. Resistance to Glycerine.

Moussu (1926) found that a portion of brain preserved its virulence at room temperature (July–August, Alfort) for 18 days, but that its pathogenic action was lost after 32 days. According to Zwick the brain of a rabbit preserved its virulence in glycerine for from 4 to 5 months, and in our experiments glycerinated virus kept in the cold room at 4° C. was still virulent after 113, 135, and in one case 161 days.

To find the best conditions for keeping the virus in the cold room the following solutions were tried. (1) Pure glycerine, (2) pure glycerine covered with a layer of sterile paraffin oil, (3) glycerine mixed with an equal part of sterile physiological saline, (4) glycerine mixed with an equal part of phosphate saline M/25, pH. 7.6. The brain of Rabbit 77A (dead of Borna disease 48 days after inoculation intracerebrally with typical lesions in the C.N.S.) was taken aseptically and divided into four equal portions, and one of the portions placed in each of the four media referred to above. At the end of a certain time a fragment of each portion was taken and an emulsion of it inoculated intracerebrally into rabbits to test its virulence. A table of results is given on page 18.

The results recorded in the table on p. 18 show that the virus may remain virulent in the cold room at 4° C. at least 113 days in a medium consisting of pure glycerine, glycerine diluted to 50 per cent. with physiological saline, or glycerine diluted to 50 per cent. with phosphate saline M/25, pH. 7.6. In two further experiments under similar conditions the virus preserved in glycerine remained virulent for 135 days and 161 days respectively.

(1) An emulsion of the brain of *Rabbit 25* (dead of Borna disease 35 days after inoculation) which had been kept in glycerine in the ice-chest for *135 days* was inoculated intracerebrally into Rabbit 145A.

	No. of days in medium.	*No. of rabbit inoculated.*	*Weight in gms.*	*Commencement of disease.*	*Duration of disease.*	*Death.*	*Lesions in C.N.S.*
Virus kept in pure glycerine	20 days	232	1,340	20th day	11 days	31st day	Intense.
	113 ,,	140A	2,020	30th ,,	10 ,,	40th ,,	,,
Virus kept in pure glycerine covered with paraffin oil	20 ,,	244	1,200	33rd ,,	12 ,,	45th ,,	,,
	113 ,,	146A	1,780	26th ,,	20 ,,	26th ,,	,,
Virus kept in glycerine mixed with equal parts of physiological saline.	22 ,,	261	1,280	23rd ,,	8 ,,	31st ,,	,,
	113 ,,	147A	1,680	26th ,,	14 ,,	40th ,,	,,
Virus kept in glycerine and phosphate saline. M/25, pH. 7.6	22 ,,	219	1,820	16th ,,	5 ,,	21st ,,	,,
	113 ,,	143A	1,780	20th ,,	6 ,,	32nd ,,	,,

Rabbit 145A. Weight 2,500 gms.

Date.	*Weight in gms.*	*Clinical observations.*
13.6.27	2,500	Normal.
22.6.27	2,500	,,
29.6.27	2,500	,,
7.7.27	2,320	First symptoms of the disease.
14.7.27	1,900	Typical symptoms of the disease in an advanced stage.
19.7.27	1,550	
20.7.27	—	Died 47 days after inoculation.

Cultures from brain. Negative.
Microscopic examination. Brain and cord showed presence of intense lesions.

(2) Virus (brain of Rabbit 100 dead of enzootic encephalo-myelitis on the 37th day after inoculation) kept in glycerine 161 days in the chest, was inoculated intracerebrally into *Rabbit 142A* on the 3.6.27.

Rabbit 142A. Weight 2,440 gms.

Date.	*Weight in gms.*	*Clinical observations.*
13.6.27	2,500	Normal.
22.6.27	2,620	,,
29.6.27	2,440	,,
7.7.27	2,040	Typical symptoms commencing.
14.7.27	1,850	,, ,, ,,
16.7.27	1,600	Died 43 days after inoculation.

Cultures from brain. Negative.
Microscopically. Characteristic and intense lesions were present in the central nervous system.

We have observed that certain of the rabbits infected intracerebrally with virus kept in glycerine succumbed to the disease at an earlier date than rabbits inoculated with an emulsion of fresh virulent brain as is indicated by the following experiment.

(3) *Rabbit 275* was inoculated intracerebrally with a virus kept in pure glycerine for 48 days on 10.2.27.

Date.	*Weight in gms.*	*Clinical observations.*
10.2.27	2,100	
19.2.27	2,150	No abnormal symptoms observed.
23.2.27	2,040	,, ,, ,,
27.2.27	1,940	,, ,, ,,
5.3.27	1,700	,, ,, ,,
7.5.27	—	Commencing paresis of the hind quarters.
9.3.27	1,520	Paresis of hindquarters more advanced.
12.3.27	—	Animal remained in corner of the cage hunched up, depressed. The paresis was still present.
13.3.27	1,260	Intense salivation. Paralysis of the hind quarters.
14.3.27	1,220	Died 32 days after the inoculation.

The microscopic examination of sections of brain and cord of Rabbit 142A revealed the presence of intense and characteristic lesions.

The following table, which shows the chain of the series of passages of the virus from rabbit to rabbit, of which Rabbit 275 forms a connecting link, demonstrates the fact that although it weighed more than 2,000 gms. it died at an earlier date than the other rabbits of the same series.

No. of Rabbit.	*Inoculated intracerebrally with*	*Interval between inoculation and death.*
100	Fresh virus.	37 days.
77A	Fresh virus from Rabbit 100.	48 ,,
275	Virus from Rabbit 77A kept in glycerine for 48 days.	32 ,,
211	Fresh virus from Rabbit 275.	42 ,,
212	,, ,, ,,	44 ,,

A similar observation has been made a number of times in the course of our experiments, and this is recorded as a typical example. Levaditi, Harvier, and Nicolau record similar findings for the virus of herpes, and this has been confirmed more recently by Perdrau.

V. Sensitiveness to Heat and Desiccation.

(a) *Heat.* Zwick and his collaborators found that cerebral emulsions heated for 5, 10, 15, 20, and 25 minutes respectively at 50° C. preserved their virulence for the rabbit. In some instances a similar emulsion heated for 30 minutes at 50° C. became avirulent. Heated for 30 minutes at 57° C. or 10 minutes at 70° C. in the water-bath the virulence of the emulsion was destroyed.

(b) *Desiccation.* Zwick found that a virulent emulsion of brain dried for 6 to 10 hours at 30° C. proved to be avirulent when inoculated 1 or 10 days after such desiccation.

VI. Action of Ultra-violet Light.[1]

We proceeded in the following manner. A homogeneous emulsion of virulent brain was centrifugalized for 5 minutes. The supernatant fluid was carefully pipetted into a small quartz flask and exposed for 5 minutes to the rays from a mercury arc. Two mercury vapour lamps (K.B.B. type, 25 amperes, 210 volts D.C.) were employed 8 inches distant. The flasks were slowly rotated during the exposure so that a fresh thin film of fluid was constantly exposed to the lamp. The flasks dipped periodically into cold water in a trough to prevent overheating during the experiment. The irradiated fluid was inoculated into the brain of a rabbit. At the same time a portion of the non-irradiated emulsion was inoculated intracerebrally into two rabbits, which served as controls. The protocols of these experiments are recorded on page 21.

The rabbit inoculated intracerebrally with the virus which had been subjected to the rays of the mercury arc, did not show any symptoms during three months, while the controls died after 23 and 34 days respectively, showing that the virus subjected to the action of ultra-violet rays (radiations of wave-lengths 5,720–2,320 A.U.) is killed in a maximum of 5 minutes.

Rabbit 163A was reinoculated, along with a control rabbit, No. 25A, with fresh passage virus 86 days later and died on the

[1] We are indebted to Dr. Eidinow of the Department of Applied Physiology (National Institute for Medical Research) for his collaboration in these experiments.

38th day. The control rabbit succumbed to the injection on the 48th day.

Irradiated emulsion.	*Controls—non-irradiated emulsion.*	
Rabbit 163A. Weight, 2,120 gms.	*Rabbit 165A. Weight, 2,010 gms.*	*Rabbit 166A. Weight, 1,900 gms.*
13.6.27. 1,940 gms. Normal.	13.6.27. 1,950 gms. Normal.	13.6.27. 1,780 gms.
22.6.27. 1,950 „ „	22.6.27. 1,900 „ „	22.6.27. 1,500 „
29.6.27. 2,000 „ „	29.6.27. 2,000 „ „	29.6.27. 1,400 gms. Typical symptoms.
7.7.27. 2,160 „ „	7.7.27. 1,490 gms. Typical symptoms.	3.7.27. Found dead 23 days after inoculation.
14.7.27. 2,300 „ „	14.7.27. 1,000 gms. Rabbit dying, killed 34 days after infection.	*Microscopic examination.* Intense lesions in the central nervous system of a characteristic type.
1.8.27. 2,650 „ „	*Microscopic examination.* Intense characteristic lesions in the central nervous system.	
15.8.27. 2,800 „ „		
28.8.27. 2,800 „ „		
5.9.27. 2,800 „ „		

VII. The Action of Hexamethylenetetramine (Urotropine).

The experiments of Moussu (1926) show that when equal quantities of a virulent emulsion of brain and a 10 per cent. solution of urotropine are mixed and kept at room temperature for 12 hours, the virus can still be demonstrated in the mixture after this time.

VIII. Action of Chloroform and Ether.

(a) *Action of Chloroform.* A thick emulsion of the brain of a rabbit dead of experimental Borna disease (Rabbit 182A, dead on the 40th day after cerebral infection) was mixed with five volumes of chloroform, and the mixture kept for 18 hours at room temperature. The fluid part of the mixture was removed by evaporation in a vacuum over sulphuric acid. The dried brain residue was powdered in a mortar and suspended in physiological saline. This suspension was then inoculated intracranially into two rabbits (234A and 235A). Rabbit 219A, which was inoculated with an emulsion of the same brain not mixed with chloroform, served as control.

Five days later both Rabbits 234A and 235A received a further inoculation with the virus which had undergone similar treatment with chloroform.

Virus treated with chloroform.		*Control rabbits—virus not treated with chloroform.*
Rabbit 235A. 2,600 gms.	*Rabbit 234A. 2,470 gms.*	*Rabbit 219A. 2,000 gms.*
5.8.27. Inoculated.	5.8.27. Inoculated.	5.8.27. Inoculated.
10.8.27. Second inoculation given.	10.8.27. Second inoculation given.	15.8.27. 2,100 gms. Normal.
15.8.27. 2,300 gms. Normal.	15.8.27. 2,450 gms. Normal.	24.8.27. 1,789 gms. Commencement of disease.
24.8.27. 2,350 „ „	24.8.27. 2,500 „ „	29.8.27. 1,580 gms. Typical disease.
29.8.27. 2,400 „ „	29.8.27. 2,550 „ „	2.9.27. Found dead 31 days after infection.
9.9.27. 2,500 „ „	31.8.27. Reinoculated with fresh virulent virus.	Passage of brain to fresh rabbit is positive.
15.9.27. 2,700 „ „	9.9.27. 2,600 gms. Normal.	*Microscopic examination* of sections of brain and cord revealed intense and characteristic lesions.
28.9.27. 2,880 „ „	15.9.27. 2,700 „ „	
	28.9.27. 2,670 „ „	

These experiments show that the virus is inactivated by contact with chloroform for 18 hours at room temperature.

(b) *Action of Ether.* The technique was similar to that employed in the above experiment with chloroform. Rabbit 231A, which received an intracerebral inoculation of ether-treated brain emulsion, was kept under observation for 5 months. It never showed any symptoms of Borna disease and gained 780 gms. in weight. The control rabbit, 219A, inoculated with non-treated brain died in 31 days of a typical infection.

NOTE.—The experiments on the effect of chloroform and ether on the virus are only preliminary. Obviously there are certain details in the technique used which will have to be controlled, and improved methods are now being employed.

IX. ACTION OF FORMALIN.

About one gramme of the brain of Rabbit 355A (which died 27 days after intracerebral inoculation with the passage virus of Borna disease) was emulsified in 15 c.cms. of a solution of formalin in physiological saline (2 : 1,000). The emulsion was rendered as homogeneous as possible, and was then placed at the temperature of the laboratory for 18 hours. Subsequently rabbits were inoculated intracerebrally with the formolized emulsion. An emulsion of the brain of Rabbit 355A in a similar dilution not treated with formalin was inoculated as a control into the brain of a rabbit.

Protocols. The inoculations were made 10.8.27.

Virus treated with formalin inoculated intracerebrally.			*Control—virus not treated with formalin.*
Rabbit 44A. 2,710 gms.	*Rabbit 45A. 2,000 gms.*		*Rabbit 356A. 2,100 gms.*
15.8.27. 2,600 gms. Normal.	15.8.27. 1,800 gms. Normal.		15.8.27. 2,050 gms. Normal.
23.8.27. 2,700 „ „	23.8.27. 1,750 „ „		23.8.27. 2,150 „ „
30.8.27. 2,640 „ „	30.8.27. 1,650 „ „		30.8.27. 1,900 „ „
5.9.27. 2,750 „ „	5.9.27. 1,800 „ „		5.9.27. 1,780 gms. Disease commencing.
11.9.27. 2,740 „ „	11.9.27. 1,950 „ „		11.9.27. 1,550 gms. Typical disease.
15.9.27. 2,800 „ „	15.9.27. 2,010 „ „		14.9.27. Found dead 36th day.
22.9.27. 2,720 „ „	22.9.27. 1,980 „ „		Typical microscopic lesions in
6.10.27. 2,740 „ „	6.10.27. 2,000 „ „		central nervous system.
12.10.27. 2,800 „ „	12.10.27. 2,000 „ „		
21.10.27. 2,780 „ „	21.10.27. 2,010 „ „		

Conclusion. The conclusion arrived at is that formalin in a concentration of 0·2 per cent. inactivates the virus after 18 hours' contact at room temperature.

X. CULTURE.

All attempts at cultivation of the virus have remained negative up to the present.

XI. SUMMARY.

From the observations of the authors quoted and our own, it appears that the virus of Borna disease possesses the properties common to those of vaccinia, herpes, rabies, and poliomyelitis which Levaditi has grouped together under the name 'ectodermoses neurotropes'. Under favourable conditions it filters through bacteria-proof filters,

although most of the virus is held back, and through a collodion ultrafilter which will allow colloidal particles of large dimensions to pass. The infectivity of the supernatant fluid cannot be removed by centrifugalization for 15 minutes at 5,400 revs. per minute. It is resistant to the action of glycerine, but sensitive to desiccation, ultra-violet light and heat. It is destroyed by ether, chloroform, and formalin. It has not been propagated outside the body.

3. TRANSMISSION OF EQUINE STRAIN TO RABBIT AND FROM RABBIT TO SHEEP, AND VICE VERSA

Moussu (1926) inoculated an emulsion of the brain of a rabbit previously infected with the virus from a horse into the anterior chamber of the eye of a horse. The animal developed symptoms 3 days after the inoculation and died in 8 days. The lesions found in the brain were very intense, infiltrative, and haemorrhagic. A rabbit inoculated with an emulsion from the brain of this horse died 4 days later.[1] This same author failed to infect horses with virulent material from rabbits by subcutaneous inoculation or *per os*. Zwick and his collaborators (1926) inoculated a horse intracerebrally with the brain of a rabbit suffering from experimental Borna disease. The virus had been passaged in this species of animal nine times. The horse fell ill 53 days after the inoculation. For 11 days it showed the typical symptoms of encephalo-myelitis and death followed 64 days after the inoculation. The lesions of the nervous system were characteristic of Borna disease. The intranuclear corpuscles of Joest-Degen were demonstrated in the ganglion cells of the Ammon's horn.

We have referred previously to the experiments of Beck and Frohböse (1926), Moussu (1926), Miessner (1926), and Ernst and Hahn (1927), which showed that the virus of encephalo-myelitis originating from sheep can be transmitted to rabbits by experimental inoculation. Moussu and Marchand (1924) succeeded in passing the disease from sheep to sheep. Zwick and his collaborators (1926) failed to transmit the disease to adult sheep by intracerebral inoculation with a strain derived from a horse and passed through rabbits. Using the same strain of virus they succeeded, however, in conferring the disease on young lambs. Death followed 88 days after the inoculation and typical cerebral lesions were revealed.

Direct inoculation from horse to lamb was also successful. The lamb showed characteristic symptoms and died 92 days after infection. Inoculation of the brain of this lamb to a rabbit gave a positive result, but inoculation of the cord gave a negative result. Beck and Frohböse (1926) did not succeed in infecting the horse by the intracerebral route with virus from sheep dead from the spontaneous

[1] The experiments of Moussu and Marchand have been criticized since his inoculated animals succumbed very early, and also because the lesions of the brain were surprisingly acute when compared with those found in the classical disease occurring spontaneously or in animals infected with the viruses isolated by the German school. We had the intention of comparing their strain with those of Zwick and Miessner, but Moussu has informed us that his strain is not now available.

disease. On the other hand, they succeeded in infecting sheep with virus obtained from horses.

From the foregoing *résumé* of the literature the following conclusions may be drawn:

(1) The virus originating from horses passaged through rabbits can be transmitted back to the horse.
(2) The virus taken directly from the horse or subsequently passaged through rabbits is pathogenic for lambs.
(3) Attempts at transmitting the disease from sheep to horses have so far been unsuccessful.

4. EXPERIMENTAL DISEASE IN THE RABBIT

I. Experimental Transmission of the Disease to the Rabbit

Moussu (1926) inoculated an emulsion of the brain of a horse dead of encephalo-myelitis into the anterior chamber of the eye of the rabbit. In one experiment three animals were inoculated by this route. One died on the 9th day; the two others survived; the rabbit which died constituted the head of the series of passages that the author continued until he obtained a 'fixed' virus which killed the rabbits infected by the intraocular route in 4 to 6 days. In another experiment using similar material one out of five animals inoculated intraocularly died 11 days after receiving the injection; the other four survived. In the majority of cases a marked excitability was a characteristic symptom. Moussu states, however, that certain rabbits die 'following an infection with a slower evolution, lasting more than a fortnight'.

Zwick inoculated rabbits intracerebrally with the cerebral substance (Ammon's horn, caudate nucleus, and cerebral cortex) taken from the brain of a horse dead of Borna disease. He observed typical symptoms of the disease after a period of about 4 weeks, and the lesions were analogous to those described previously in the horse. Passage from rabbit to rabbit was effected, the period of incubation after inoculation being about 3 weeks.

Zwick and his collaborators (1926) succeeded in infecting rabbits with the virus from 16 out of 21 cases of horses dead of Borna disease which was verified histologically. The incubation period of the disease in such rabbits infected by the intracerebral route was from 3 to 4 weeks. Death ensued 8 to 14 days after the appearance of the characteristic symptoms.

Beck and Frohböse (1926) also infected rabbits with the virus from horses and sheep. Miessner (1926) with sheep virus, and Ernst and Hahn (1927) with viruses from horses, sheep, and cattle.

II. Authors' Observations.

The virus of encephalo-myelitis of equine or ovine origin isolated by the German workers does not become 'fixed' when passaged through rabbits. The period of incubation varies from 15 to 50 days, and we have also observed recurrent forms of the disease in rabbits.

We have inoculated more than 200 rabbits by the intracerebral route with the virus originating from horses or sheep. In Table I the period of incubation and the time between the onset of the disease and death is recorded for a total of 50 rabbits used for passaging a virus originally obtained from a horse and sent to us by Professor Zwick.

TABLE I.

Number of rabbit.	*Weight in gms.*	*Commencement of disease.*	*Duration of the disease.*	*Death.*	*Lesions in C.N.S.*
25	2,160	21st day	14 days	35th day	Intense.
22	2,000	20th ,,	4 ,,	24th ,,	Positive.
24	1,420	21st ,,	19 ,,	40th ,,	,,
10D	2,500	27th ,,	10 ,,	37th ,,	Intense.
11D	1,000	17th ,,	10 ,,	27th ,,	,,
*93A	1,520	7th ,,	1 ,,	8th ,,	Slight.[1]
68A	1,150	20th ,,	5 ,,	25th ,,	Intense.
50A	1,620	40th ,,	13 ,,	53rd ,,	,,
77A	1,570	38th ,,	10 ,,	48th ,,	,,
80	1,350	21st ,,	7 ,,	28th ,,	,,
51A	1,350	20th ,,	8 ,,	28th ,,	,,
34	2,000	43rd ,,	14 ,,	57th ,,	,,
298	820	25th ,,	14 ,,	39th ,,	,,
275	2,100	25th ,,	7 ,,	32nd ,,	,,
202	1,050	29th ,,	3 ,,	32nd ,,	,,
282	1.040	27th ,,	4 ,,	31st ,,	,,
244	1,200	33rd ,,	12 ,,	45th ,,	,,
261	1,280	23rd ,,	8 ,,	31st ,,	,,
232	1,340	20th ,,	11 ,,	31st ,,	,,
219	1,820	16th ,,	5 ,,	21st ,,	,,
295	1,300	9th ,,	10 ,,	19th ,,	Very intense.
211	1,580	31st ,,	11 ,,	42nd ,,	,,
212	1,400	31st ,,	13 ,,	44th ,,	,,
220	1,500	31st ,,	8 ,,	39th ,,	,,
222	1,540	35th ,,	13 ,,	48th ,,	,,
224	1,150	32nd ,,	13 ,,	45th ,,	,,
226	1,150	24th ,,	4 ,,	28th ,,	Positive.
70	1,700	20th ,,	2 ,,	22nd ,,	Intense.
56	1,400	20th ,,	6 ,,	26th ,,	,,
61	1,500	24th ,,	6 ,,	30th ,,	,,
251	1,280	18th ,,	9 ,,	27th ,,	,,
237	1,680	18th ,,	13 ,,	31st ,,	,,
255	1,920	35th ,,	7 ,,	42nd ,,	,,
256	1,860	32nd ,,	15 ,,	47th ,,	,,
30	1,120	28th ,,	8 ,,	36th ,,	,,
44	1,350	26th ,,	7 ,,	33rd ,,	,,
58	1,000	27th ,,	7 ,,	34th ,,	Positive.
*291	950	No sympt.	—	15th ,,	Slight.[1]
85B	1,500	32nd ,,	6 ,,	38th ,,	Very intense.
*43	1,020	No sympt.	—	12th ,,	Slight.
60	1,800	30th ,,	7 ,,	37th ,,	Intense.
62	1,610	22nd ,,	17 ,,	39th ,,	,,
271	1,220	31st ,,	10 ,,	41st ,,	,,
*80	820	No sympt.	—	7th ,,	Slight.
269	2,200	19th ,,	2 ,,	21st ,,	Positive.
*775	1,780	No sympt.	—	13th ,,	Positive.[1]
296	2,900	26th ,,	7 ,,	33rd ,,	Intense.
277	2,100	13th ,,	11 ,,	24th ,,	,,
78S	1,700	24th ,,	8 ,,	32nd ,,	,,
116A	1,700	18th ,,	4 ,,	22nd ,,	,,

[1] When the brain of these rabbits was passaged, positive results were obtained. Rabbits inoculated with passage of virus from the brain of Rabbit No. 93A died in

Of 50 rabbits inoculated in the brain:

23 died between 21 and 33 days after inoculation.

6 died in less than 21 days.

21 died in more than 33 days.

Only exceptionally did the rabbit die in less than 3 weeks when infected by the intracerebral route. The detailed histopathological study of each case showed that the rabbits dead 7 to 8 days after inoculation had minimal infiltrative lesions in the central nervous system. The presence of virus in the brain was proved by subsequent passage and in all cases complete autopsies were made to exclude the possibility of death from other causes.

The animals which died between 21 days and 57 days presented the characteristic lesions in the nervous system, which are described in full in the chapter dealing with the histopathology of the disease. The intensity of the lesions was not in direct relationship with the duration of the malady. As has also been observed by Zwick the incubation period was longer in larger animals. Generally, rabbits weighing less than 1,500 gms. were more susceptible to infection than older rabbits. Excluding the five animals in Table I marked with an asterisk, all of which died in 15 days or under, the average time which elapsed between the intracerebral inoculation and the death of the animals in our experiments was 20 days in rabbits of less than 1,500 gms. and 36 days in rabbits over this weight at the time of infection.

TABLE II.

Number of rabbit.	*Weight in gms.*	*Commencement of disease.*	*Duration of disease.*	*Death.*	*Lesions in C.N.S.*
164	1,220	21st day	8 days	29th day	Intense.
18	1,240	26th ,,	9 ,,	35th ,,	,,
246	1,380	28th ,,	10 ,,	38th ,,	,,
234	1,420	7th ,,	—	7th ,,	Slight.
243	1,420	20th ,,	2 ,,	22nd ,,	Intense.
235	1,500	22nd ,,	9 ,,	33rd ,,	,,
262	2,220	40th ,,	8 ,,	48th ,,	,,
Q30	3,640	23rd ,,	12 ,,	35th ,,	,,
10	2,140	18th ,,	9 ,,	27th ,,	,,
91	1,620	19th ,,	8 ,,	27th ,,	,,
274	1,770	18th ,,	10 ,,	28th ,,	,,
28	1,900	23rd ,,	14 ,,	37th ,,	,,
86	1,800	20th ,,	12 ,,	32nd ,,	,,
63	1,600	20th ,,	8 ,,	28th ,,	,,
162A	2,180	21st ,,	12 ,,	33rd ,,	,,
167A	2,420	22nd ,,	7 ,,	29th ,,	,,
178	1,770	25th ,,	9 ,,	34th ,,	,,
207A	840	19th ,,	2 ,,	21st ,,	Well marked.
181A	2,100	26th ,,	6 ,,	32nd ,,	,,
69	1,670	26th ,,	7 ,,	33rd ,,	,,
180A	1,890	27th ,,	12 ,,	39th ,,	,,
179A	2,220	26th ,,	6 ,,	32nd ,,	,,

Table II sets forth similar observations in the case of 22 rabbits

53 days; from No. 291, in 38 days; from No. 778 in 21 days. Intense lesions were found in the C.N.S. of these latter rabbits.

inoculated with a strain of ovine origin kindly sent to us by Professor Miessner. The average time between inoculation and death was 33 days for the 15 rabbits weighing more than 1,500 gms. and 28 days for the 6 rabbits weighing less than 1,500 gms.

III. Symptomatology of the Disease in the Rabbit.

Observations have been made on over 200 rabbits. During the first 2 to 4 days following the injection the weight of the animal decreases slightly, to return later to the normal. Once the period of traumatic shock has subsided the animal puts on weight and no morbid symptoms are seen during 15 to 20 days. Subsequently it becomes slow in its movements and appears depressed; the weight decreases progressively and the first characteristic symptom develops. When the rabbit is placed on its side it makes efforts to recover its feet, beating the air with its hind legs before eventually recovering the normal position. While the depression referred to above suggests modifications in the meninges and cerebrum, the symptoms described later point to changes in the spinal cord.

The animal assumes a characteristic attitude in the cage with the head in the angle formed by two walls; it appears somnolent and the somnolence lasts till the end of the disease. The symptoms of nervous origin become intensified gradually; among these are those of amaurosis. When the animal is allowed to run towards an object it runs into it as if it had not seen it. Grinding of the teeth is observed, sometimes with increased salivation. There is paresis of the ears, which fall to the right and left of the head. The head itself is depressed. When the animal is placed at the edge of a table it hangs its head over the edge below the level of the rest of the body. Trismus may occur. The symptoms of a myelitic character become exacerbated. Taken from the cage and placed on its side, the rabbit makes vain efforts to rise. At this stage its position at rest is characteristic; it remains hunched up in a corner, the head is dropped as if it was no longer capable of supporting it (Fig. 1), and sometimes the back is humped. The muscles of the back become soft and flaccid. Attempts to resist with the hind legs when the animal is held by the skin of the back are feeble or absent. Finally paralysis of the hind quarters occurs, which spreads later to the fore-limbs. The animal ceases to feed, either from loss of appetite or difficulty in deglutition, and loses weight'. In certain cases the loss of weight may be the dominant feature of the disease. Very often rabbits at the end of the disease have lost nearly half of their original weight (Charts I and II).

We have never observed excitement in our experimental animals, but always depression.

The study of the blood has given inconstant results. In certain animals a slight hyperleucocytosis has been observed with a slight increase in polymorphonuclears. In others the leucocytosis 16,000 to 18,000 per c.mm. was accompanied by lymphocytosis. In the

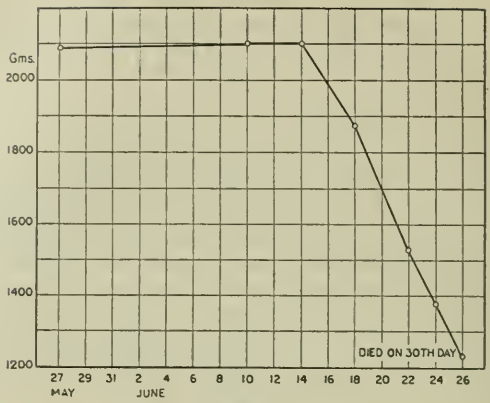

Chart I.

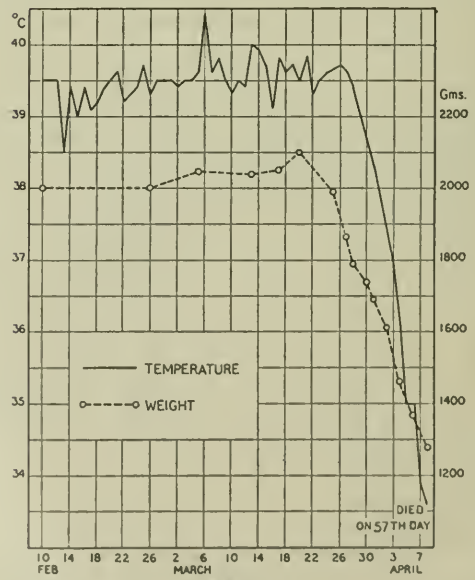

Chart II.

terminal stage a marked increase in polymorphonuclears is the rule. The number of erythrocytes remains unchanged, and they show no morphological changes.

Chart III shows the parallelism between the augmentation of the number of leucocytes per c.mm. and the number of lymphocytes obtained from the leucocytic count. This modification of the number of leucocytes is not constant.

No fever exists during the course of the disease in the rabbit. This fact was noted constantly when the temperature of a series of rabbits

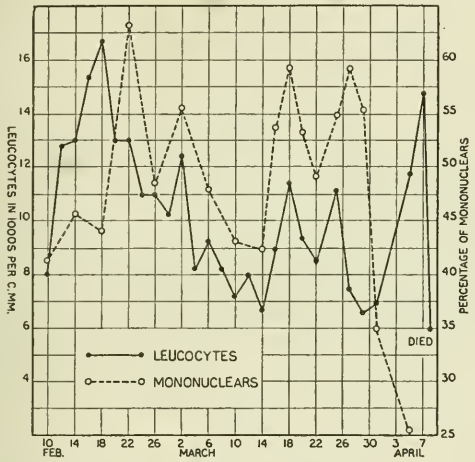

Chart III.

inoculated intracerebrally was taken daily at the same hour. Death takes place in coma—the temperature being hypothermic (35° C.–34° C.: see Chart II).

IV. Routes by which Rabbits can be Infected.

Intracerebral.

Intracerebral inoculation produces the disease in a constant manner, followed by death.

Intrathecal.

Beck (1925) infected by introducing virus intrathecally, and we have also succeeded in infecting rabbits by this route.

Experiment 1. Rabbit 207, weighing 780 gms. was inoculated intrathecally in the lumbar region with 0·5 c.cm. of a virulent emulsion of brain diluted 1 : 10 in physiological saline. Forty-three days

after the inoculation the condition of the animal was such that when taken out of the cage it walked with its legs spread out from the body. Paresis of the hind quarters was well marked, and increased gradually. The animal wasted and died 66 days after the inoculation. Examination of the brain revealed the presence of characteristic lesions, and a passage of this brain to a healthy rabbit gave a positive result.

Sciatic Nerve.

Introduction of several drops of a virulent emulsion of the virus into the sciatic nerve did not infect animals with encephalo-myelitis in the experiments of Moussu (1926), and Zwick and his collaborators (1926). We have, however, succeeded several times in producing Borna disease in rabbits inoculated by this route.

Experiment 2. The right sciatic nerve of Rabbit 208, weighing 850 gms., was exposed by incision and 2 or 3 drops of a virulent emulsion of the brain of a rabbit infected with encephalo-myelitis was injected into the substance of the nerve-trunk. The point of introduction of the needle was seared to prevent the exit of fluid into the surrounding tissues. The operation was carried out aseptically and the incision healed by first intention. The animal showed no morbid symptoms for 35 days and put on weight, reaching 1,350 gms. Subsequently it became prostrate, wasted progressively, and showed marked ineo-ordination, which became accentuated later; the animal died on the 48th day. The brain was proved to be bacteriologically sterile, and no lesions were found which might serve to explain the cause of death, other than those in the central and peripheral nervous system. These were of an intense character, and were found in the brain, in the spinal cord (cervical, thoracic, dorsal, and lumbar regions), and also in the inoculated nerve.

Emulsions from the brain and also the dorso-lumbar part of the spinal cord were inoculated into the brain of fresh rabbits. These inoculations produced the disease, showing that the virus was present both in the brain and cord of rabbits inoculated into a peripheral nerve.

Rabbit 270 was also inoculated into the sciatic nerve by the same method as recorded above for Rabbit 208. This rabbit (270) showed, 65 days after the inoculation, paralysis of the leg into the sciatic nerve of which virus had been inoculated. Paralysis of the hind quarters followed, with grinding of the teeth, and other typical symptoms. The animal was found dead on the 78th day. Lesions were demonstrated throughout the nervous system (brain, mesencephalon, cord in all regions, inoculated nerve, the sciatic of the opposite side (non-inoculated), as well as in the brachial nerves).

The detailed description of these lesions will be given later.

Rabbit 276 was inoculated into the right sciatic with the same technique as before. The leg on the side of inoculated nerve became useless after 28 days, and the animal died 8 days later with lesions in the central and peripheral nervous system.

Anterior Chamber of Eye.

Moussu (1926) and also Zwick (1926) have shown that it is possible to infect the rabbit by inoculation of a virulent emulsion of the brain of the horse into the anterior chamber of the eye, and we have confirmed the possibility of infection by this route.

Rabbit 206 received several drops of the supernatant fluid from a virulent emulsion of brain into the anterior chamber. The point of inoculation was carefully seared. During the period immediately following the inoculation a coagulum of fibrin could be seen in the eye, but this was absorbed later. The animal died 23 days after the inoculation, and lesions characteristic of Borna disease were found in the central nervous system; these, however, were not very acute.

The control rabbit inoculated intracerebrally with the same emulsion died in the average time with well-marked lesions in the central nervous system.

Rabbits 86s and 89s inoculated in the anterior chamber with an emulsion containing virus fell ill 29 and 36 days respectively after infection, and died on the 34th and 51st day with typical symptoms in the central nervous system. The control rabbit (intracerebral route) died 37 days after inoculation.

A fourth rabbit inoculated intraocularly with the virus survived without having shown any symptoms.

Thus, of four rabbits inoculated in the anterior chamber three became infected and died of the disease, while the fourth showed no morbid symptoms and survived. These results are in accordance with those of Zwick who had five positive results in six attempts to infect rabbits by the intraocular route.

No macroscopic modification of the cornea followed the introduction of the virus, but microscopically there was slight infiltration with lymphocytes between the corneal laminae. In one case an interstitial infiltration with mononuclear cells of the optic nerve was found.

Corneal Scarification.

Zwick (1926) has stated that infection by corneal scarification causes the disease only rarely. We have inoculated four rabbits by scarification of the cornea; none became infected, nor was there any visible keratitis. However, one of the rabbits (Rabbit 81s) which was subsequently reinoculated intracerebrally with a virulent emulsion of brain 110 days later remained well, whereas a control rabbit which had received a cerebral inoculation with the same emulsion died 48 days later after showing typical symptoms of the disease, and with the characteristic lesions. The corneal inoculation may have rendered the rabbit refractory to infection by the intracerebral route.

Conjunctival Sac.

Instillation of a virulent emulsion into the conjunctival sac of the eye produced no effect.

Nasal Mucosa.

The nasal mucosa appears to be a possible portal of entry of the virus, and Joest (1927) has suggested that natural contagion in the

horse is effected by this route. The results of attempts by Zwick (1926), Beck and Frohböse (1926) to infect rabbits by this route have, however, been inconstant.

Scarified Skin.

Zwick applied a virulent emulsion of brain to the scarified skin without result. Our results [1] confirm those of Zwick. As, however, the experiments of Flexner and Amos (1917) with poliomyelitis, and of Levaditi and Nicolau (1922, 1923) with herpes and neurovaccinia show that previous injection of substances like physiological saline, bouillon, or normal serum into the brain may increase susceptibility, we introduced physiological saline either into the brain or intrathecally in rabbits which had received an application of the virus on to the depilated, shaved, and scarified skin. The animals prepared by the inoculation of saline *intrathecally* did not show any symptoms of the disease, while those which had been subjected to an irritation of the *brain* with saline subsequently contracted the disease, and died. Typical lesions were found in the central nervous system, and passage of the brain to new rabbits by the intracerebral route gave positive results. This experiment shows that when the nervous system is in a state of special receptivity due to the diminution of the normal power of defence, infection can take place from the skin. The virus probably reached the brain by way of the intercostal nerves, thus being protected from the action of leucocytes circulating in the blood. This interpretation is supported by the following experiments. Four rabbits were inoculated with 1·5 c.cms. of a centrifugalized emulsion of virulent brain into the marginal vein of the ear. Two of the rabbits inoculated intravenously received simultaneously 0·3 c.cm. of physiological saline into the brain, but neither of these animals contracted the disease, nor did the two which received only the intravenous inoculation. Two months later the immunity of these four rabbits was tested by intracerebral inoculation, and all proved susceptible to infection. The virulence of the emulsion used to infect the four rabbits intravenously was proved by the intracerebral inoculation of two controls. So that it would appear that the virus was destroyed in the blood-stream, or in the tissues before it reached the brain.

Our further attempts to infect by the intravenous route gave us negative results, but Zwick (1926) succeeded exceptionally when 4 intravenous injections were given at intervals. Positive results have also been obtained by Ernst and Hahn (1927). Usually, however, they found that repeated inoculations by the intravenous route instead of conferring the malady produced solid resistance. This will be discussed in the chapter dealing with immunity.

Subcutaneous.

Subcutaneous inoculation may, exceptionally, lead to a fatal encephalo-myelitis. Zwick and his collaborators (1926), who employed repeated injections of the virus, produced the disease with a greater

[1] At no time did the skin show any macroscopic changes which could be attributed to the virus.

frequency. They obtained similar results by inoculation of the virus *intraperitoneally*; *intramuscular* injections with the virus did not confer the disease in their experiments.

Intratesticular.

According to the latter investigators the introduction of virus by the intratesticular route did not produce infection in rabbits, but in our experiments it has done so.

Rabbit 209, weighing 1,850 gms., was inoculated under anaesthesia into both testicles with a virulent emulsion of the brains of four rabbits dead of experimental Borna. The dilution of the emulsion of brain substance in physiological saline was 1 : 20. During a period of 43 days the animal showed no morbid symptoms and its weight increased to 2,540 gms.; then, without other symptoms, wasting commenced; 19 days later the weight had fallen to 1,860 gms. (680 gms. loss), and inco-ordination with slight paresis of the hind quarters was noticed. Paresis became accentuated and other symptoms characteristic of the disease became manifest. On the 71st day after the inoculation, 20 days after the first loss of weight was recorded and 8 days after the first clinical symptom, the animal died, weighing only 1,480 gms. The lesions found in the central nervous system were characteristic and were especially intense in the lumbar region. The topography of the lesions in the cord indicated that the virus had spread from the point of inoculation to the brain through the cord centripetally. The intranuclear inclusions of Joest-Degen were found.

The passage of the brain and cord of *Rabbit 209* to fresh animals gave positive results, indicating the presence of virus in both. The control rabbit inoculated intracerebrally with the emulsion of brain which served to infect *Rabbit 209* died 32 days after inoculation.

Rabbit 289, weighing 1,720 gms., was inoculated into the right testicle with a virulent emulsion of brain. During 60 days it put on weight, reaching 2,320 gms. This weight was maintained for 14 days, when wasting began. On the 90th day after inoculation paresis of the hind quarters was observed. The animal died 105 days after inoculation.

Rabbit 273 inoculated into the right testicle at the same time showed no symptoms and survived, while the control rabbit inoculated intracerebrally died after 37 days with the typical symptoms and lesions characteristic of the disease.

Intratracheal.

We have made two unsuccessful attempts to infect rabbits by *intratracheal* inoculation. In the first attempt two rabbits received each 0·3 c.cm. of a thick emulsion of brain containing virus into the trachea, which had been exposed by incision; both these rabbits survived without having shown any symptoms, while the control which had received an *intracerebral* inoculation with the same virus succumbed to the infection. In the second experiment four young rabbits, between 670 and 860 gms., were inoculated. Each received 0·5 c.cm. of a thick emulsion of the brain of a rabbit, dead of Borna disease,

diluted 1 : 5. Kept under observation more than six months they maintained their normal state of health, more than doubling their weight. The controls of this experiment inoculated intracerebrally died of a typical encephalo-myelitis on the 42nd and 44th day after injection.

Per Os.

Attempts at infecting rabbits per os are of special interest for the interpretation of natural infection in horses, cattle, and sheep. Zwick and his collaborators (1926) succeeded in infecting rabbits by mixing virulent brain with the food. He refers to this as infection by the intestinal route, but as the virus was administered by the mouth with the food, the pre-existence of small traumatic lesions in the mouth might permit the implantation of the virus. As infection by the nasal mucosa has been shown to be possible, one cannot exclude the possibility that infection took place by the buccal mucous membrane, especially when one considers the existence of nervous tissue immediately below the mucous membrane covering the tongue (Manouelian and Viala, 1926). Supposing the virus to have been implanted in such nervous tissue, it is quite easy to conceive how it might ultimately reach the brain.

Attempts to infect rabbits by *cohabitation* have been unsuccessful.

5. AUTHORS EXPERIMENTS ON THE TRANSMISSION OF THE DISEASE TO MONKEYS (*MACACUS RHESUS*), AND SYMPTOMS OCCURRING IN THESE ANIMALS

Monkey M. 1. A fine specimen of *Macacus rhesus* weighing 3,800 gms. was kept under observation during 16 days prior to inoculation. The animal's temperature varied very little.

On 15.3.27 it was inoculated intracerebrally under anaesthesia with 1·5 c.cms. of a virulent emulsion of the brain of a rabbit diluted 1 : 5. Two control rabbits were inoculated intracerebrally at the same time as the monkey; they developed typical symptoms on the 28th and 31st day, and died of Borna disease on the 42nd and 44th day.

Between 15.3.27 and 11.5.27, a period of 57 days, the monkey showed no symptoms and the temperature remained normal.

15.3.27. Weight 3,800 gms. Temperature 38·9° C. Received inoculation with the brain emulsion from Rabbit 275.

11.5.27. Fifty-seven days after inoculation the monkey appeared depressed. Temperature 38·4° C. Slight diarrhoea.

12.5.27. Same condition.

14.5.27. Temperature 38·5° C. No diarrhoea. Less lively than usual, appeared to prefer to remain with the back to the light (photophobia ?).

15.5.27. Condition unchanged.

16.5.27. Photophobia well marked. The monkey hid its head under the straw of the cage and would not move when disturbed. It allowed itself to be caught easily and defended itself when approached almost exclusively with the left hand and foot. Slight paresis of the right arm was detected. The pupils were equal and reacted normally to light.

18.5.27. Weight 3,280 gms. Temperature 38·2° C. Animal feeding normally. It remained in a corner of the cage with the head dropped like that of a

rabbit ill from Borna disease. It allowed itself to be caught easily, offering but little resistance and with the left arm only. It was found possible to introduce the thermometer into the rectum without holding the legs, which fell practically inert. When the monkey was put on the ground it moved much more slowly than normally, dragging the right leg, which showed paresis. Paresis was less evident in the left leg. The animal could grip the cage with the left hand, the only limb which preserved its normal function. The right leg and arm did not grip or gripped only badly. The animal attempted to climb, but fell exhausted by the effort. It was roused with difficulty. There appeared to be no trouble in preserving equilibrium. The animal appeared to be able to see. The diarrhoea was replaced by constipation.

19.5.27. Temperature 38·5° C. Same symptoms as on the preceding day. On opening the cage the monkey did not move or try to get out. When taken out and left to run it fell on the right side, of which the paralysis was more accentuated. When the monkey was held by the skin of the neck, the legs, which showed a marked flaccidity, fell inert without any resistance (Fig. 2). If a finger was presented, the monkey gripped it with the left hand only, the right hand showed paralysis of the flexors of the digits.

20.5.27. Temperature 38·5° C. Same condition.

21.5.27. Temperature 38·1° C. Weight 3,280 gms. Animal still feeding. When taken out of the cage it was found to tire quickly, and after a feeble effort at escaping it remained on the ground immobile for several minutes.

23.5.27. Temperature 38·0° C. Motor disturbances were accentuated. Monkey remained hunched up in a corner of the cage (Fig. 3).

24.5.27. Temperature 37·5° C. Same condition.

25.5.27. Temperature 37·3° C. Complete paralysis of the legs. When making movements, it supported itself especially with the left arm and dragged the paralysed legs. For the most part it preferred to remain hunched up in the corner of the cage.

26.5.27. Found procumbent. Weight 3,180 gms.

27.5.27. Temperature 36·5° C. Animal procumbent, the respirations were irregular and infrequent. Incontinence of urine, no grinding of the teeth, no ocular symptoms, no hypersalivation, both legs paralysed. As the animal was dying it was killed by means of chloroform at 6 o'clock in the evening 73 days after the inoculation, 16 days after the first symptoms were observed.

Autopsy of Monkey 1.

The dura mater, pia mater, and the brain substance appeared normal.

The cord was slightly hyperaemic, but no haemorrhagic areas were found.

The buccal epithelium and tongue showed desquamation of the epithelium and redness.

The parotid gland. Normal in aspect.

Lung. Normal.

Spleen, liver, and gall bladder. Normal.

Kidney. Congested in both the cortical and medullary zones.

Adrenal. Normal.

Peritoneal cavity, bladder, and intestines. Normal.

Cultures made from the brain, spleen, and blood proved to be bacteriologically sterile.

Note. A study of the blood, made during the course of the infection, indicated no decided changes; the leucocytic formula remained normal, except for a slight increase in the number of polymorphonuclears in the last stages of the disease.

Passages into rabbits made with emulsions from various parts of the central nervous system of this monkey gave positive results (see p. 48).

An emulsion of the brain of M. 1 was likewise inoculated into two monkeys of the same species by the intracerebral route (Monkey M. 2 and M. 3) on the 28.5.27.

Monkey M. 3 (*Macacus rhesus*). Weighed 3,150 gms.

During a period of 71 days after the inoculation, the animal remained free from all morbid symptoms, and the temperature remained normal. On the 72nd day the monkey was found procumbent, although the day before there was no obvious illness. It was paralysed in the legs and arms, and it could not assume an upright position. Slight ptosis of the right eyelid was observed. The temperature was subnormal. The following day the respirations were irregular, gasping, and moist râles were heard. As the animal was in convulsions it was killed. The findings on autopsy were the same as for Monkey M. 1. Rabbits inoculated with emulsions of various parts of the nervous sytem died of Borna disease with characteristic lesions.

Monkey M. 2 (*Macacus rhesus*). The evolution of the disease in this monkey was entirely different.

28.5.27. Animal inoculated. Weight 3,200 gms. Temperature 38·5° C. During a period of 33 days after the inoculation the blood, the body-weight, and the temperature curve showed no marked changes, and there was no evidence of any symptoms.

30.6.27. Temperature 38·2° C. Weight 3,100 gms. When a stick was given to the animal it could not seize it with the right hand, and if irritated it defended itself with the left hand. The monkey was able to run and climb normally.

1.7.27 and 2.7.27. No change in condition.

3.7.27. In addition to the paresis of the right arm the animal showed lassitude and appeared less agile.
From the 3.7.27 to the 11.7.27. the condition remained unchanged.

14.7.27. Forty-seven days after the inoculation. Weight 2,790 gms. When taken out of the cage the animal ran and climbed with difficulty. The paresis of the right arm was ameliorated, but there was paresis of the legs, more accentuated on the left side. The left arm also showed slight paresis.

18.7.27. The left eye was closed completely, due to ptosis of the upper eyelid (Fig. 4). The face was drawn to the right side. The paresis of the arm and leg on the left side was now easily discernible. If the animal when seated was gently pushed it fell on to the left side. It uttered a plaintive cry from time to time. No lesions could be seen on the cornea or conjunctiva.

20.7.27. Marked excitation was observed. The animal appeared to have hallucinations. It threw itself at imaginary objects, attempted to bite frequently, and struggled and fell as if in a fit. There was nearly complete paralysis of the left side and ptosis of the left eyelid.

22.7.27. The symptoms appeared to be ameliorated. The animal was much calmer and the eye could be opened partially.

25.7.27. Eye nearly completely open. Animal still aggressive, uttered cries from time to time, paralysis less noticeable.

28.7.27. Eye appeared normal, and the condition of the monkey practically normal.

1.8.27. Ptosis of the right eyelid. Slight paresis of the hind quarters, which, however, was not sufficient to prevent the monkey from climbing. It became fatigued easily, however, and remained in a corner of the cage crying out from time to time.

3.8.27. Paralysis of the right side of the face (see Fig. 5) was observed. Animal could still run and climb.

5.8.27. Same condition. Weight 3,170 gms.

8.8.27. Paralysis of face diminished. Animal irascible.

12.8.27. Spasmodic contractions of muscular groups of the back and shoulders were the only signs of the animal being other than normal.

13.8.27. Left eye deviated to the internal canthus. Tendency to remain hunched up.

14.8.27. Animal decidedly ill. Head hanging over on to right shoulder: internal strabismus still present and left pupil dilated. Right eye mobile. Pupil of this eye reacted to light. Nystagmus present. The animal had periods of excitement.

15.8.27. Monkey uttering cries with a low feeble raucous voice. Mouth opened after showing dragging to the right side. Internal strabismus on the left side, pupil on this side also dilated. Nystagmus exacerbated. Paralysis of muscles of left shoulder, head falling over on to the right shoulder.

17.8.27 and 21.8.27. Condition unchanged.

27.8.27. Until 27.8.27 animal appeared normal. On this date the animal had complete aphonia (probably paralysis of the recurrent nerve). The head hung over the right side and the monkey showed signs of cerebral disorder. Strabismus was less, face was drawn to the right side, and there was frequent spasmodic contraction of the facial muscles ('tic). The pupils were unequal in size. The animal carried out movements of mastication without the teeth coming in apposition, for this reason it fed with difficulty. Deglutition was not carried out easily, and the animal appeared to have difficulty in orientation. Taken out of the cage it could not co-ordinate its movements in the direction desired.

29.8. 27. Weight 2,900 gms. Animal still made movements of mastication continually, and twitching of the muscles of the mouth was present. There was internal strabismus, as well as aphonia and weakness of the muscles of the neck on the left side. The monkey remained hunched up, or had periods of excitement which were increased by noises, movements, &c.

31.8.27. Condition unchanged.

2.9.27. Weight 2,820 gms. Spastic contractions of divers muscular groups were produced by noises. The tongue was drawn to the right side. The animal fed no more owing to the impossibility of swallowing. 'Tic' of the mouth persisted, also the strabismus and the inequality of the pupils.

4.9.27. The left eye appeared practically normal and the pupils equal in size. The animal, however, still trembled at times and bit at imaginary objects; frequent 'tic' was observed with aphonia. The animal, however, fed well.

9.9.27. The condition of the animal had not changed.

16.9.27. The cry appeared more normal, ocular disturbances were absent, although 'tic' and champing of the jaws was still observed. The head was hung over the right shoulder, and the animal showed increased salivation.

20.9.27. The condition of the animal was unchanged; to a certain degree the syndrome in this monkey might be compared to that exhibited by a man affected with post-encephalitic Parkinsonism.

22.9.27. Same condition. Weight 3,000 gms.

5.10.27. Up to this date the monkey remained in a similar state, this being the 130th day since the inoculation. At this time the animal was inoculated with the virus of polio-myelitis. The result is given in the chapter dealing with immunity.

These three monkeys showed three different forms of the disease. In all three cases the incubation period was very long, but the duration of the morbid symptoms varied. Monkey 3 had an acute attack which lasted only 2 days. In Monkey 1 the evolution was slower, the time between the onset of symptoms and death being 16 days. Monkey 2 had recurrent attacks during a period of 97 days (see subsequent history, p. 80).

The virus after passage through monkeys had not lost its virulence for the rabbit or guinea-pig.

In the chapter dealing with histopathology the lesions in the nervous sytem will be described in full. We may, however, anticipate here by the statement that we found lesions of an intense character in the brain, and of a more discrete character in the cord. Lesions were also found in the spinal ganglia, posterior nerve-roots, and peripheral nerves. Both clinically and pathologically the disease may be described as an encephalo-myelitis complicated with a ganglio-radiculitis and peripheral neuritis. The virus when introduced into the brain evidently spreads not only to the cord, but also to the peripheral nerves, for not only have we found lesions in these, but we have also been able to demonstrate the presence of virus.

Discussion.

The pathogenicity of the virus for the monkey, and the clinical features presented in this animal, raise the question of the relation between enzootic encephalo-myelitis of domestic animals and polio myelitis in man. There are marked resemblances in the clinical aspects as well as in the alterations in the cord and spinal ganglia in the two diseases. The former virus is, however, pathogenic for the rabbit, while the latter is generally considered not to be so. The incubation period of experimental encephalo-myelitis in the monkey is longer than in the disease produced by the virus of polio-myelitis.

Another question is whether the virus of encephalo-myelitis is pathogenic for man, and if such is the case, whether some human disease of the nervous system at present of unknown origin may possibly be due to it. It is a common observation in clinical medicine that exposure to cold may determine an attack of facial neuritis or sciatica. Conceivably, such attacks might be the expression of an unrecognized infection by some virus, akin to enzootic encephalo-myelitis, which in the first place caused only slight, if any, general symptoms of disease and then proceeded down into the peripheral nerves, where it remained latent until the added factor of cold determined the local incidence of paralysis or pain. Similar views may be argued with regard to herpes zoster, recurrent herpes, or the so-called peripheral forms of epidemic encephalitis. An analogous action of cold was recorded by Pasteur, when certain rabbits that were resistant to the inoculation of an attenuated rabies virus, at once showed paralytic symptoms after exposure to severe cold.

The curious epidemic at Lille, studied by David and Dekester

(1926), has suggested that a certain form of sciatica, at least, is an infectious disease; and there are many human cases on record of myelitis associated with peripheral neuritis, apparently of a contagious type, in some of which, as in the instance of the child described by Péhu and Dechaume (1927), there were in the peripheral nerves inflammatory lesions closely resembling those described by us in monkeys and rabbits infected with Borna disease.

We give below a *résumé* of Péhu and Dechaume's case in some detail because the symptoms resemble in certain respects those which our Monkey M. 2 showed after inoculation with the virus of Borna disease. The child, 20 months old, was in perfect health up to the day when it showed some lassitude three months before going to hospital. The temperature did not exceed 36·5° C. Two months later it could no longer walk and had lost power in the arms. At the time of entering the hospital it showed flaccidity and paralysis in the lower extremities without Babinski's sign. There was also slight paresis of one arm. The following day the child collapsed although not losing consciousness. There were no cerebral symptoms, nor vomiting, neither did somnolence exist, but the pulse was rapid and irregular. The condition lasted 11 days and the child died suddenly without convulsions. The case was diagnosed as a peripheral form of epidemic encephalitis, referred to as *pseudo-myelitic*.

On autopsy there were no macroscopic changes. On microscopic examination of sections, discrete lesions, for the most part exudative, were found in the cord, especially in the lumbar region. Perivascular infiltrations occurred in the brain. No neuronophagia was recorded, and the spinal ganglia were not examined. The lesions found in sections of the median nerves, sciatic nerve, and posterior tibial nerves were comparable to those described by us in the sciatic and brachial nerves of rabbits and monkeys inoculated intracerebrally with the virus of Borna disease (see pages 61 and 67).

Péhu and Dechaume suggested that the presence of lesions in the peripheral nerves might be coexistent with the presence of virus. In the case of Borna disease we have proved that the presence of virus is coexistent with the existence of lesions in the peripheral nerves (see page 45).

6. PATHOGENICITY OF THE VIRUS OF ENZOOTIC ENCEPHALO-MYELITIS FOR THE GUINEA-PIG, RAT, MOUSE, AND FOWL

I. Guinea-pig.

The introduction of virus intracerebrally into guinea-pigs may produce the disease. The incubation period varies in individual cases and death is inconstant. Zwick and his collaborators (1926) were the first to transmit the disease to guinea-pigs, and to make passages in series from brain to brain. Some guinea-pigs proved to be resistant to infection. According to their experiments death followed infection in

from 3 weeks to 13 months. They also succeeded in infecting rabbits with the virus passaged through guinea-pigs.

In Table III we give the period of incubation and the duration of the disease in a batch of guinea-pigs which in our experiments proved to be susceptible to the virus inoculated intracranially.

TABLE III.

No. of guinea-pig.	*Weight in gms.*	*Commencement of disease.*	*Death.*	*Lesions in brain.*
85E	470	73rd day	83rd day	Intense.
84E	400	107th ,,	132nd ,,	,,
94E	500	23rd ,,	114th ,,	,,
95K	500	58th ,,	65th ,,	Discrete.
93K	480	40th ,,	58th ,,	Average.
98K	450	57th ,,	64th ,,	,,
99K	580	18th ,,	19th ,,	Intense.
100K	560	58th ,,	182nd ,,	,,

We have also made experiments to determine the relative susceptibility of guinea-pigs to infection. Forty-five guinea-pigs of about the same size (400–600 gms.) were divided into three lots of 15.

Lot A were injected with an emulsion of the brain of a rabbit dead of Borna disease diluted 1 : 10.

Lot B were injected with the same emulsion diluted 1 : 100.

Lot C were inoculated with the emulsion diluted 1 : 1,000.

The results were as follows:

In Lot A all the guinea-pigs died after showing typical symptoms of the disease, 52, 58, 74, 133, 134, 139, 139, 141, 141, 143, 148, 150, 153, 156, and 176 days after the inoculation. Lesions characteristic of enzootic encephalo-myelitis were found in sections of the brain and spinal cord of these animals; moreover, the corpuscles of Joest-Degen were demonstrated.

TABLE IV.

Lot A.

Dilution of emulsion of brain inoculated.	*No. of guinea-pig.*	*Animal died.*	*Observations.*
1 : 10	1A	52nd day	Intense lesions in central nervous system.
,,	2A	58th ,,	,, ,, ,, ,,
,,	3A	74th ,,	,, ,, ,, ,,
,,	4A	133rd ,,	,, ,, ,, ,,
,,	5A	134th ,,	Very intense lesion in C.N.S.
,,	6A	139th ,,	Discrete lesions in C.N.S.
,,	7A	139th ,,	Intense lesions in C.N.S.
,,	8A	141st ,,	,, ,,
,,	9A	141st ,,	Mild lesions in C.N.S.
,,	10A	143rd ,,	Intense lesions in C.N.S.
,,	11A	148th ,,	,, ,,
,,	12A	150th ,,	,, ,,
,,	13A	153rd ,,	Very intense lesions in C.N.S.
,,	14A	156th ,,	Intense lesions in C.N.S.
,,	15A	176th ,,	,, ,,

In Lot B two of the guinea-pigs succumbed to an intercurrent infection. The thirteen others died 58, 105, 130, 130, 135, 137, 138, 140, 140, 140, 147, 149, and 153 days after infection.

TABLE V.

Lot B.

Dilution of emulsion of brain inoculated.	*No. of guinea-pig.*	*Animal died.*	*Observations.*
1 : 100	1B	58th day	Intense lesions in central nervous system.
,,	2B	105th ,,	Slight lesions in C.N.S.
,,	3B	130th ,,	Very intense lesions in C.N.S.
,,	4B	130th ,,	Intense lesions in C.N.S.
,,	5B	135th ,,	,, ,,
,,	6B	137th ,,	,, ,,
,,	7B	138th ,,	,, ,,
,,	8B	140th ,,	,, ,,
,,	9B	140th ,,	Slight lesions in C.N.S.
,,	10B	140th ,,	Intense lesions in C.N.S.
,,	11B	147th ,,	,, ,,
,,	12B	149th ,,	,, ,,
,,	13B	153rd ,,	,, ,,
,,	14B	5th ,,	Accidental death.
,,	15B	12th ,,	,, ,,

In Lot C five of the animals died of an intercurrent infection, the ten remaining died 70, 90, 96, 101, 140, 141, 141, 147, 149, and 150 days after intracerebral inoculation.

TABLE VI.

Lot C.

Dilution of emulsion of brain inoculated.	*No. of guinea-pig.*	*Animal died.*	*Observations.*
1 : 1,000	1c	70th day	Discrete lesions in central nervous system.
,,	2c	90th ,,	Intense lesions in C.N.S.
,,	3c	96th ,,	,, ,,
,,	4c	101st ,,	,, ,,
,,	5c	140th ,,	,, ,,
,,	6c	141st ,,	,, ,,
,,	7c	141st ,,	,, ,,
,,	8c	147th ,,	,, ,,
,,	9c	149th ,,	Very intense lesions in C.N.S.
,,	10c	150th ,,	,, ,, ,,
,,	11c	5th ,,	Accidental death.
,,	12c	8th ,,	,, ,,
,,	13c	8th ,,	,, ,,
,,	14c	8th ,,	,, ,,
,,	15c	11th ,,	,, ,,

As in the case of the guinea-pigs of Lot A, the symptoms in guinea-pigs of Lot B and C were characteristic, and sections made from the

central nervous system showed the typical changes produced by the virus of Borna disease in other animals.

These results, while demonstrating the variation in individual susceptibility, also point to the fact that resistance to infection in the guinea-pig is not so marked as it appeared to be from the results obtained by Zwick, since of thirty-eight guinea-pigs inoculated in our experiments thirty-eight succumbed to the disease (the seven guinea-pigs dead from other causes are not included).

The virus passaged through guinea-pigs still preserved its pathogenicity for the rabbit.

The disease in the guinea-pig is similar to that of the rabbit. After a variable period the animal appears depressed, there is marked somnolence, and abstention from food. Characteristic nervous symptoms follow, those indicating affection of the cord being especially well marked. The syndrome is as described in the rabbit. The hind legs become paralysed (Fig. 6) and the fore legs are involved later (Fig. 7). The loss of weight is less marked than in the case of the rabbit.

From the four following experiments the susceptibility of the guinea-pig after the virus is inoculated would appear to be diminished by a simultaneous inoculation of the same material intramuscularly.

An emulsion of virulent brain originating from a rabbit dead of experimental Borna disease was inoculated into the brain of eight guinea-pigs, and at the same time 1 c.cm. of the same emulsion was inoculated into the quadriceps group of muscles of four of them. The results of these four experiments are tabulated below:

Table VII.

	No. of guinea-pig.	*Guinea-pig died.*	*Lesions in the brain.*
I. Inoculated into the brain	95K	65th day	Discrete.
Inoculated into the brain and muscle.	97B	131st ,,	Intense.
II. Inoculated into the brain	93K	58th ,,	Of average intensity.
Inoculated into the brain and muscle	91B	116th ,,	,, ,,
III. Inoculated into the brain	99K	19th day	Intense.
Inoculated into the brain and muscle	96B	Survived	
IV. Inoculated into the brain	98K	64th day	Of average intensity.
Inoculated into the brain and muscle	200B	184th ,,	Intense.

This observation is comparable to that of Ernst and Hahn (1927), who found that when rabbits inoculated intracerebrally with virulent emulsion received, either at the same time or subsequently, injections of virus into the veins, they did not develop a fatal encephalitis.

Attempts at infecting the guinea-pig by intradermal inoculation of an emulsion of virulent brain into the metatarsal pad (following the technique used by Waldmann and Pape (1921) in foot-and-mouth disease, and by Gildemeister and Herzberg (1925) in experimental herpes) did not succeed. The guinea-pigs inoculated varied in weight from 100 gms. to 750 gms., and were kept under observation for over seven months, but no symptoms were seen at any time during this period.

II. Rat.

Zwick, Seifried, and Witte (1926) infected rats with the virus of Borna disease by intracerebral inoculation. Death supervened 40, 53, and 62 days respectively, after infection. Some rats showed no symptoms and survived. The virus passaged through the rat had not lost its pathogenicity for the rabbit.

In our experiments large rats appeared to be more susceptible to the disease than young animals. Four rats (three old and one young) were inoculated intracerebrally with an emulsion of the brain of rat No. I which died 67 days after infection. (Typical lesions of Borna disease were found in sections of the brain of rat No. 1.) The three large rats died 22, 37, and 74 days respectively after inoculation. They all developed typical symptoms, and sections of the brain showed the presence of characteristic lesions microscopically. The young rats kept under observation for six and a half months remained perfectly normal.

The control rabbit inoculated with the same emulsion of brain from Rat 1 died on the 27th day of a typical infection.

Subsequently four rats (two large and two small) were inoculated with an emulsion of one of the brains of one of the large rats mentioned above (that dead on the 37th day). The two older rats died on the 40th and 82nd day after infection, while the two younger animals survived 124 days, succumbing later to an intercurrent infection. In all, we have inoculated twenty-eight rats; of these only the older rats contracted the disease.

The symptoms in the rat are similar to those in the guinea-pig. They commence with motor disturbances, inco-ordination, and difficulty in maintaining equilibrium. Paralysis, coma, cachexia, and death follow later.

Up to the time of writing we have succeeded in making at least four passages in this species. The rats in the series died 67, 37, 82, and 47 days respectively, after inoculation, showing that the course of the disease in the rat is as variable as in the guinea-pig. It would appear that virus passaged through rats when inoculated intracerebrally into rabbits produced the disease after a shorter incubation period than when the virus was passaged in series through rabbits.

In sections of the brain of our experimental rats the corpuscles of Joest-Degen were found. Zwick (1926), however, failed to find them in the brain of rats inoculated with the virus of Borna disease.

III. Mouse.

We have been able to infect mice by the intracerebral route, but this species of rodent is apparently less susceptible to the infection. As in the rat, age appears to have an important bearing on the susceptibility of the mouse to the disease. Mice weighing more than 20 gms. generally contracted the disease and died, while smaller mice survived without showing symptoms. In our experiments mice died on the 37th, 52nd, 81st, and 126th day respectively, after inoculation.

These mice wasted considerably, walked with tortoise-like movements, and showed other motor disturbances. Typical lesions were demonstrated in the brains of the mice and the intranuclear '*inclusions*' of Joest-Degen were present in the Ammon's horn.

IV. Fowl.

Zwick, Seifried, and Witte (1926) found the fowl to be susceptible to intracerebral inoculation. In one case the incubation period was 37 days, and death followed 15 days later. Passage from fowl to rabbit gave a positive result.

7. ANIMALS WHICH HAVE BEEN FOUND TO BE RESISTANT TO INFECTION WITH THE VIRUS OF ENZOOTIC ECEPHALO-MYELITIS

I. Dog.

According to Zwick, Seifried, and Witte (1926) the dog appears to be resistant to infection with the virus of Borna disease. This fact obviates up to a certain point confusion with the virus of rabies. A greater number of experiments require to be done, however, before the dog can be definitely classed among the animals resistant to infection.

II. Pigeon.

These same authors demonstrated that the pigeon is resistant to intracerebral infection with the virus.

III. Ferret.

We inoculated six ferrets, three young and three adults, by the intracerebral route and kept them under observation for seven months, but no morbid symptoms developed.

8. DISTRIBUTION OF THE VIRUS OF BORNA DISEASE IN THE ANIMAL BODY

I. Passage of Virus through the Placenta.

Ernst and Hahn (1927) showed that the virus is capable of passing the placenta of the mare and infecting the foetus during intra-uterine life. In two cases the virus was demonstrated by inoculation

of rabbits intracerebrally with the brain of foals born of mothers ill with enzootic encephalo-myelitis. Further, they demonstrated lesions characteristic of Borna in the sections of the brain of both the foals and the mothers in these cases.

II. Distribution of the Virus in Various Organs and Tissues

Zwick, Seifried, and Witte (1926) tested four samples of blood from infected rabbits, three samples of blood, two of spleen, two of kidney, and two of liver, from horses ill from Borna disease, but failed to find the virus. Ernst and Hahn (1927), on the other hand, proved the blood to contain the virus during some stages of the illness of a rabbit suffering from Borna disease.

Similar apparently contradictory results have been obtained in experimental infections produced by other filterable viruses where the virus may sometimes be found in the blood, e.g. rabies, vaccinia, herpes, and foot-and-mouth disease.

Ernst and Hahn (1927) found the vitreous body of the eye infective after a rabbit had been inoculated intracerebrally. The virus has also been demonstrated by Zwick and his collaborators (1926) in the submaxillary salivary gland in inoculated rabbits.

III. Presence of Virus in the Peripheral Nerves of Rabbits Inoculated Intracerebrally.

The present writers have demonstrated the virus in the peripheral nerves of rabbits infected by the intracerebral route in which infiltrating lesions in the nerve occurred.

Experiment 1. A portion of both sciatic nerves taken from 1 cm. below their emergence from the greater sciatic foramen to the popliteal region was removed aseptically from Rabbit 130A, which died 50 days after intracerebral inoculation. An emulsion of these two portions of sciatic nerve was made in physiological saline and inoculated into the brain of Rabbits 213A and 214A.

Rabbit 213A.

Weight 2,000 gms.

14.7.27. Intracerebral inoculation with emulsion of sciatic nerve.
1.8.27. Animal normal. 2,000 gms.
7.8.27. Animal normal.
15.8.27. Commencement of paresis of the hind quarters. 2,000 gms.
24.8.27. Typical symptoms of the disease. 1,600 gms.
29.8.27. Animal very ill. 1,350 gms.
29.8.27. Died the 46th day after the inoculation.

Autopsy. All organs macroscopically normal. Cultures from the brain negative.
Sections. Intense lesions of a characteristic type in the central nervous system. The corpuscles of Joest-Degen were also demonstrated.

Rabbit 214A.

Weight 2,100 gms.

14.7.27. Intracerebral inoculation with an emulsion of the sciatic nerve.
1.8.27. Normal. 2.250 gms.
7.8.27. Animal normal.
15.8.27. Typical symptoms of the disease. Head depressed; placed on its side the animal showed the characteristic myelitic syndrome. 1,800 gms.
20.8.27. Animal very ill. 1,600 gms.
22.8.27. Found dead 39 days after the inoculation.

Autopsy. No lesions in organs. Cultures from the brain negative.
Sections. Intense lesions characteristic of Borna disease were demonstrated throughout the central nervous system.
Passage. The brain of this rabbit was passaged to Rabbit 291A.

Rabbit 291A.

24.8.27. Intracerebral inoculation with an emulsion of the brain of Rabbit 214A.
15.9.27. Typical symptoms of the disease. 1.590 gms.
21.9.27. Found dead the 28th day. 1,150 gms.
Characteristic intense lesions were found in the central nervous system, and the corpuscles of Joest-Degen were demonstrated.

The two rabbits died after showing typical symptoms of the disease. Lesions of a characteristic type were demonstrated throughout their central nervous system, and moreover, the virus was demonstrated in their brain.

Experiment 2. In a second experiment the virus was sought for also in the brachial nerve of a rabbit which had succumbed 31 days after inoculation into the brain. The nerves were removed aseptically and emulsified in sterile mortars. The emulsions were then inoculated intracerebrally into rabbits. The results of the inoculation are given below.

(1) *Brachial Nerve.*

Rabbit 36A. Weight 2,870 gms.

15.9.27. Inoculated intracerebrally with an emulsion of the brachial nerve of Rabbit 252A.
22.9.27. No symptoms. 2,850 gms.
28.9.27. Animal normal. 2,700 grms.
6.10.27. Animal normal. 2,620 gms.
10.10.27. Commencement of paresis. 2,350 gms.
12.10.27. Typical symptoms of the disease. 2,150 gms.
14.10.27. Animal died 30th day after inoculation. 1,800 gms.

Autopsy. All organs appeared normal.
Cultures of the brain. Negative.
Microscopic examination of sections of the brain and other parts of the central nervous system showed the presence of typical lesions.

(2) *Sciatic Nerve.*

Rabbit 35A. Weight 1,200 gms.

15.9.27. Inoculated intracerebrally with an emulsion of the sciatic nerve of Rabbit 252A.
22.9.27. Animal normal. 1,050 gms.
28.9.27. Animal normal. 1,020 gms.
6.10.27. Animal normal. 1,100 gms.

10.10.27. Commencement of paresis. 1,150 gms.
12.10.27. Typical symptoms of the disease. 1,050 gms.
14.10.27. Typical symptoms of the disease. 960 gms.
15.10.27. Found dead 37 days after infection.

Autopsy. All organs normal.
Cultures of the brain. Negative.
Typical lesions were found on microscopical examination of sections from the central nervous system.

This second experiment shows that when the virus is introduced into the animal organism by the intracerebral route, it may subsequently be found in the brachial as well as the sciatic nerves.

In a third experiment *Rabbits 27A and 30A* were inoculated intracerebrally with an emulsion of the sciatic nerve taken as in experiment 1.

Rabbit 27A.

Rabbit 27A. Weight 1,690 gms.

6.9.27. Date of inoculation.
15.9.27. Animal normal. 1,750 gms.
22.9.27. Animal normal. 1,900 gms.
28.9.27. Animal normal. 1,870 gms.
6.10.27. Animal normal. 1,880 gms.
10.10.27. Commencement of the disease. 1,850 gms.
12.10.27. Typical symptoms of the disease. 1,700 gms.
15.10.27. Animal in agonal stage of death. 1,450 gms. Found dead later, 39th day after infection.

Autopsy. No macroscopic lesions.

Rabbit 30A.

Rabbit 30A. Weight 1,750 gms.

6.9.27. Date of inoculation.
15.9.27. Animal normal. 1,750 gms.
22.9.27. Animal normal. 1,820 gms.
28.9.27. Animal normal. 1,900 gms.
6.10.27. Animal normal. 2,000 gms.
10.10.27. Slight paresis of hind quarters. 2,050 gms.
12.10.27. Slight paresis of hind quarters. 2,000 gms.
21.10.27. Typical symptoms of the disease present. 1,900 gms.

All these eight rabbits inoculated intracerebrally with emulsions of either the brachial nerve or sciatic nerve of rabbits dead of experimental enzootic encephalitis contracted the disease and died. The presence of lesions, and of the corpuscles of Joest-Degen in the central nervous system of these eight rabbits, as well as the positive passage made with the brain of one of them, indicates that the virus of Borna disease generalizes into the peripheral nervous system centrifugally. The lesions occurring in the peripheral nerves are described later (see p. 61).

IV. Distribution of the Virus in various Organs and Tissues of the Monkey.

We have studied the distribution of the virus in the animal organism of *Monkey M. 1* (*Macacus rhesus*) (see p. 34) which died of Borna disease 73 days after infection.

Experiment 1. Two rabbits were inoculated intracerebrally with emulsions of the cerebrum, medulla oblongata, spinal cord (dorso-lumbar), parotid, spleen, testicle, blood, and adrenal glands.

The results are set forth in Table VIII.

Table VIII.

Organ.	*No. of rabbit.*	*Weight in gms.*	*First appearance of disease.*	*Death.*	*Lesions.*	*Passage.*
Cerebrum	115A	1,700	22nd day	37th day	+	+
	126A	2,100	23rd ,,	30th ,,	+	+
Medulla oblongata	117A	2,280	19th ,,	27th ,,	+	+
	123A	1,200	22nd ,,	37th ,,	+	+
Spinal cord	109A	1,640	20th ,,	27th ,,	+	+
	124A	1,640	20th ,,	30th ,,	+	+
Parotid gland	121A	2,060	Dead accidentally	12th ,,	0	–
	130A	1,860	34th day	51st ,,	+	+
Spleen	125A	2,400	Death due to other causes 14th day	—	0	–
	127A	1,000	Survived			
Testicle	131A	2,100	Survived			
	132A	1,700	Survived			
Adrenal gland	119A	2,000	30th day	40th ,,	+	+
	133A	1,100	26th ,,	55th ,,	+	+
Heart Blood (defibrinated)	120A	2,060	Death due to other causes		0	–
	112A	1,800	Survived			

The virus was demonstrated in the brain, medulla oblongata, spinal cord, parotid, and adrenal glands; it was not demonstrated in the spleen, testicle, or blood.

The observations were repeated with the organs of *Monkey M. 3* (see p. 36), and the results are given in Table IX.

TABLE IX.

Organ.	*No. of rabbit.*	*Weight in gms.*	*First appearance of disease.*	*Death.*	*Lesions.*	*Passage.*
Cerebrum	252A	1,370	20th day	31st day	+	No passage made.
	258A	1,850	20th ,,	26th ,,	+	,, ,,
Spinal cord	255A	1,400	20th ,,	30th ,,	+	,, ,,
	261A	3,020	20th ,,	28th ,,	+	,, ,,
Parotid gland	265A	1,950	—	Accidentally 11th day	±	Negative.
	266A	3,390	Survived			
Adrenal gland	249A	2,070	—	15th ,,	±	,,
	267A	2,790	—	17th ,,	−	,,
Ovary	259A	2,520	Survived			
	271A	1,690	—	Accidentally 10th day		
Bone marrow	262A	1,750	Survived			
	264A	1,850	—	8th day	−	Negative.
Liver	250A	2,350	—	6th ,,		
	268A	2,320	Survived			
Kidney	254A	720	,,			
	270A	2,150	,,			
Heart blood defibrinated	269A	1,750	,,			
	272A	1,740	—	Accidentally 3rd day	−	
Lung	251A	3,290	Survived			
	263A	1,970	,,			
Mesenteric gland	273A	750	,,			
	274A	1,540	,,			

In this monkey the virus was found in the brain and spinal cord only, and could not be demonstrated in the ovary, spleen, bone marrow, liver, kidney, the blood, the lung, the mesenteric glands, adrenal glands, or parotid gland.

9. ELIMINATION OF THE VIRUS FROM THE ANIMAL ORGANISM

As is the case with the viruses of herpes, rabies, &c., the pathogenic agent of enzootic encephalo-myelitis is eliminated by the *saliva* and *nasal secretions*. The results of the German school (Zwick, Seifried, and Witte, 1926; Ernst and Hahn, 1926) are in agreement with regard to this point. The *urine* of animals ill from the disease has always proved avirulent.

10. HISTOPATHOLOGY OF BORNA DISEASE

I. Horse.

Siedamgrotzky and Schlegel (1896) described the disease as a 'serous leptomeningitis'. Both Johne (1896) and Ostertag (1900 et seq.) failed to find lesions in the brain or its coverings, and considered the disease to be an intoxication of the central nervous system by bacterial toxins. Dexler (1900) refers to Borna disease as a disseminated encephalo-myelitis with leucocytic infiltration around the vessels and in the nerve substance. Oppenheim (1907) considered it to be an acute localized meningo-encephalitis of a non-purulent nature, the meninges being more especially affected. Joest and Degen (1909), who made a more detailed study of the histo-pathology of Borna disease of the horse, regarded it as an acute meningo-encephalomyelitis, non-purulent in character, with perivascular infiltrations by lymphocytes in the cord and brain. Cellular 'inclusions' were also found in the ganglion cells of the Ammon's horn, in the hippocampus, and sometimes also in other regions of the brain. These 'inclusions' they described are within the nucleus, round in shape, and arranged sometimes in pairs. They stain red with Mann's or Lentz's stain, and have often an unstained halo around them. Their dimensions vary from the limit of visibility to the size of a nucleolus. Heydt (1914) was able to confirm the findings of Joest and Degen in every detail. Moussu and Marchand (1924) (see also thesis of Moussu, 1926) did not find the intranuclear corpuscles described by Joest and Degen.

The cases investigated by Moussu and Marchand were of an acute haemorrhagic type and polymorphonuclear leucocytes were found in the lesions in large numbers. They described an agglomeration of infiltrating mononuclear cells surrounding the nerve-cells in the brain, producing in certain cases deformation of these cells by compression of the cell membrane. These authors did not record actual neuronophagia.

Zwick, Seifried, and Witte (1924), Beck and Frohböse (1926), and Ernst and Hahn (1927) confirmed the work of Joest and Degen (1909) Zwick and his collaborators emphasize the fact that the German workers have never found degenerative lesions of the neuron nor neuronophagia in any region of the nervous system where they were searched for.

II. Cattle.

The similarity of the disease in cattle and horses, as indicated by a study of the lesions in the central nervous system with the presence of virus in these lesions, has been mentioned by Ernst and Hahn (1927). They demonstrated the corpuscles of Joest-Degen in the brain of cattle dead of the disease. A detailed description of the lesions has also been given in the thesis of Moussu (1926). He states that 'the process in the central nervous system in enzootic encephalomyelitis of cattle consists of a diffuse polio-encephalitis with a predominance of lesions in the cerebral cortex, of which the deeper layers

are more particularly affected. There are similar lesions in the basal ganglia of the cerebral hemisphere, in the peduncle, in the medulla oblongata, and even in the spinal cord; but these lesions are always less marked than those in the cerebral cortex. This encephalitis is accompanied sometimes by perivascular lesions and diffuse capillary haemorrhages. It is easy to understand that these lesions may lead to rapid death when affecting the medulla oblongata'.

III. Sheep.

Priemer (1925), Beck (1925), Moussu (1926), Miessner (1926), and Ernst and Hahn (1927) made a study of the lesions in the central nervous system in sheep dead of the disease. They came to the conclusion that the tissue changes were analogous to those produced by the virus of the equine type. Beck was the first to demonstrate the intranuclear corpuscles of Joest-Degen in the nerve-cells of the brain of sheep dying from the spontaneous disease.

No observer has described any departure from the normal in the histology of other organs either in sheep, horses, or cattle.

IV. Experimental Borna Disease in Horses and Sheep.

The lesions described in the nervous system of horses and sheep infected experimentally are identical with those found in the spontaneous disease.

V. Rabbits Infected Experimentally.

The first description of lesions in the central nervous system in rabbits infected with the virus of encephalo-myelitis was given by Moussu and Marchand (1924). These authors were apparently working with a virus which differed from those isolated by workers in Germany; and, while this difference may possibly be accounted for by an increased virulence, their description of the lesions has remained unique and unconfirmed up to the present. It is as follows:

'In rabbits which die soon after the inoculation, the alterations are those of an acute meningo-encephalitis with a predominance of lesions in the pia mater. The meninges are infiltrated with immature cells of which a large number contain eosinophile granules. These same cells can be found around intracerebral vessels (cortical or subcortical). There are alterations of the choroid plexus, and epithelium of the ventricles; there is infiltration of the subependymal zone and the pyramidal zones are much altered. The lesions of the cerebellum are identical with those of the brain, while the alterations in the vessels of this region are also as intense as in the meninges.

No bacteria were found, nor intranuclear inclusions. In animals which died at a later stage the lesions were less intense. The lesions predominate in the anterior region of the brain and are those of a subacute encephalo-myelitis. The inflammatory lesions of the meninges are only observed in the septum and in the spaces between the convolutions. The same may be said of perivascular infiltrations.

Embryonic cells ("cellules embryomaires") are present, containing eosinophile granules. There is an inflammation of the ependyma with subependymal lesions.

In the olfactory lobe one finds small inflammatory areas. The cellular lesions are well marked, but less intense than in the preceding form. In the cerebellum one finds several areas of periarteritis situated in the white matter. No bacteria or inclusions can be found.'

Zwick, Seifried, and Witte (1926), like Beck and Frohböse, Ernst and Hahn (1927), and Miessner (1927), who have been interested, in passing, in the histopathology of the central nervous system of rabbits infected experimentally, devote only a few lines to the question. In general, the summary of their findings may be given as follows: Macroscopically, apart from the brain and cord, which appeared to be hyperaemic, all the organs preserved their normal aspect.[1] A microscopic study of the lesions in the brain revealed a slight meningitis with mononuclear cells; more or less infiltration of the cerebral cortex and the Ammon's horn with lymphocytes; perivascular infiltrations, especially in the small and middle-size vessels; and the presence of intranuclear corpuscles in the large ganglion cells of the Ammon's horn. Zwick and his collaborators, as well as other investigators who have studied the disease in Germany, have never observed degenerative processes in the nerve-cells nor recorded neuronophagia. Zwick found perivascular infiltrations in the spinal cord in cases which had paresis or paralysis.

The summary given above records briefly the observations made by other workers whose attention has been directed particularly to the brain, while the participation of the spinal cord in the pathological process has been referred to only exceptionally. There is no published work on lesions of the nerve-roots, spinal ganglia, and peripheral nerves.

VI. Authors' Observations.

A. *Rabbit.*

(1) *Macroscopical and Microscopical Findings in Diverse Organs.*

Our observations have been made on animals infected either with the strain of virus originating from horses (Zwick) or that originating from sheep (Miessner). On post-mortem examination in the majority of cases, a congestion of the meninges which may sometimes be intense is found. In other cases the aspect of the nervous system may be normal.

Sometimes the *stomach* presents the lenticular haemorrhages

[1] Ernst and Hahn (1927) described haemorrhagic lenticular formations in the mucosa of the stomach of rabbits dead of Borna disease. We have found these lenticular formations (see Fig. 9) in the majority of cases in which rabbits had a prolonged paralytic phase and a long agonal stage. Under the microscope they appeared to be haemorrhages following upon an autodigestion of the stomach mucosa. They did not appear to have any definite structure, nor are they specific for experimental enzootic encephalomyelitis, since we have found them also in herpetic encephalitis and other morbid conditions.

referred to above, but we have found that these are not a specific reaction to the virus (Fig. 9). In the larger number of cases examined in detail (more than thirty rabbits) the *kidneys* showed a marked congestion. This hyperaemia was not limited to the cortical zone, but affected the medullary zone to the same extent. Sections from such kidneys showed that there were small multiple haemorrhages in the region of the glomerulus, as well as in the collecting and convoluted tubules. These extravasations formed sometimes actual haemorrhagic areas. The epithelium lining the renal tubules was normal, there were no infiltrative processes peri- or intratubular. The condition may be described as renal congestion; not a true nephritis.

On microscopic examination the *parotid* showed occasionally small areas of infiltration composed of lymphocytic elements surrounding certain of the striated canaliculi; at the same time the cytoplasm of certain of the cells of the acini had become oxyphilic when stained by Mann, while the nucleus appeared oedematous and took up an abnormal eccentric position in the cells. Although many sections of the parotid of rabbits were examined, these lesions were found only occasionally. Without presuming that they were produced by the action of the virus, it should be mentioned that they coincided with the presence of virus in this organ. In rabies and distemper, oxyphilic corpuscles, intra- or extra-cellular, staining red by Mann's method, have been described as concomitant with the presence of virus in the parotid. No actual corpuscles have been found by us in the parotid of animals infected with the virus of enzootic encephalomyelitis.

In the medullary zone of the *adrenal* small accumulations of lymphocytic elements were occasionally seen. The *lung*, the *liver*, the *spleen*, the *testicle*, and the *ovary* appear macro- and microscopically normal.

Characteristic lesions of an intense nature are found only in the nervous system. We may class these in two categories, (1) *infiltrative* and (2) *degenerative*. Both types of lesions may be met with in the brain, mesencephalon, cerebellum, spinal cord, and spinal ganglia. In the nerve-roots and peripheral nerves (sciatic and brachial nerves examined) only infiltrative lesions have been found.

(2) *Lesions in the Central Nervous System.*

(a) *The Brain.* The *pia mater* is infiltrated with mononuclear leucocytes, varying in individual cases. In some areas only a trace of this infiltrative process may be seen, while in others three or four layers of infiltrative cells occur. They are especially marked in the region of the meningeal vessels as well as in the spaces between the convolutions, and may form actual meningeal plaques. The infiltrating elements are lymphocytes, plasma cells, and large mononuclears. Vessels of the pia mater are often surrounded by 'cuffs' constituted by mononuclear leucocytes (Fig. 10). The most intense lesions of the meninges are generally found at the base of the brain. In sections

cut at right angles to the surface of the brain vessels passing from the meninges into the cortical substance surrounded by lymphocytic 'cuffing' have often the aspect of septa (Fig. 11). In the cortex diffuse infiltration of lymphocytes accompanied by proliferation and mobilization of the neuroglial cells occurs. Especially in the hippocampus (the so-called 'elective zone' in herpetic encephalitis of the rabbit), the neuron degenerates, the nucleus swells, the chromatin becomes rarefied, collects towards the periphery of the nucleus, and in its place appear small oxyphilic globules which may be at the limit of visibility or may reach the size of a nucleolus. Some of the neuroglial cells of this region appear to undergo the same degenerative process. It is similar to, although less intense than that described by Levaditi, Harvier, and Nicolau (1922) in experimental herpetic encephalitis in the rabbit, leading to the formation of *encephalitic neurocorpuscles*, and is not of a specific nature. The oxyphilic degeneration of the nucleus may lead to the formation of larger corpuscles surrounded by a halo, morphologically identical with those described as specific in Borna disease by Joest and Degen (1909). The nucleus may react in the same way to other causes. Even the halo is not wanting in the figures given by Levaditi, Harvier, and Nicolau. We regard this phenomenon as possibly the result of the action of a karyotropic virus, the degenerated karyoplasm fusing round the pathogenic agent. These nuclear lesions may be found in all regions of the brain, although the German writers have described their presence only in the *Cornu Ammonis*, in which, indeed, they are more constantly found. They may be single or in pairs surrounded or not by a characteristic halo. When sections are stained with Mann's stain or toluidin blue-eosin, the intranuclear corpuscles are stained rose or red, while the nucleolus is more of a violet tint. They occur also in the pyramidal cells of the cerebral cortex and even in the neuroglial cells (large granulo-adipose cells of the hippocampus).

In the Ammon's horn newly formed capillaries are sometimes seen. The vessels of this region appear dilated, gorged with blood, and their adventitia infiltrated with several layers of mononuclear leucocytes. Plasma cells are abundant in the process of perivascular infiltration. We have never found such a large number in rabies of the dog or rabbit, in poliomyelitis of monkeys, in human encephalitis, in chronic herpetic encephalitis of rabbits, or in the vascular lesions produced by the presence of the so-called '*Encephalitozoon cuniculi*'. Small lymphocytes may also be found in large numbers in these perivascular infiltrations, but large mononuclears cells are also present to a less extent. The presence of polymorphonuclear leucocytes is exceptional.

Sometimes in the thickness of the 'cuffing' are degenerated lymphocytes or plasma cells. The nucleus of these degenerated cells has become intensely oxyphilic. It is reduced in size and condensed in 'blocks' without any definite structure. When Mann's stain is used, the protoplasm, in the case of the degenerated plasma cells, stains rose; the unchanged elements stain blue. Here and there in the

mass of the infiltrating cells rare fragments of degenerated chromatin can be found, probably arising from degenerated mononuclear leucocytes or pyknosed polymorphonuclear cells. The intensity of the infiltration varies. Sometimes the perivascular 'cuffing' is constituted by one, two, or three layers, while in other cases a massive nodule resembling a gumma may be found with a small vessel in the centre. These alterations occur not only in the vessels of the Ammon's horn, but in all the regions of the central nervous system where 'cuffing' may be found.

In the *Cornu Ammonis* areas of mononuclear infiltration are seen between the ganglion cells, in the row of fusiform cells, or in the chain of small granular cells (Fig. 12). Neuroglial cells of neoformation may also participate in this infiltrative process.

We have never found true neuronophagia at this site, but we have encountered a curious phenomenon, the exact nature of which we had some trouble in determining. In a section of brain examined under the oil immersion we found in one of the large ganglion cells of this region stained with toluidin blue-eosin a species of 'cyst' in the interior of the protoplasm (Figs. 13, 14, 15, 16). The rest of the cell preserved its normal aspect. The 'cyst' compressed the nucleus and pushed it towards the periphery of the cell, forming a marked depression in the nuclear membrane. The diameter of this 'cyst' was about 7 μ. It was marked off from the cytoplasm of the neuron by a membrane, and contained six basiphilic granules of equal size, placed symmetrically at its periphery.

On subsequent minute examination of the cells of the Ammon's horn we found these 'cysts' again in several preparations. They have been found only in this region and always in the protoplasm of the cell. Their diameter varied from 5 μ to 9 μ. During the search we have seen occasionally plasma cells, the nucleus of which had undergone degeneration, which in its appearance recalled this intracellular 'cyst'; the karyoplasm was condensed into several small round intensely chromatophilic granules, apparently attached at equal distances to the nuclear membrane. The presence of a protoplasmic circle around this formation removed from our minds the supposition that we were dealing with a '*microsporidian cyst*', and showed distinctly that it was a degenerated infiltrating cell (Pl. I, Fig. 3). The staining reactions indicated that the cystic formations were the degenerated nuclei of the plasma cells which had penetrated into the interior of the large cells of the Ammon's horn. We have found such bodies in approximately 5 per cent. of cases examined. They have never been met with in the brain of normal rabbits, or in the brain of rabbits which have succumbed to infection with the viruses of herpes, rabies, or vaccinia.

We have described these formations in detail, since the elucidation of their nature required extended observations. Many control animals were examined to ensure that we were not dealing with a spontaneous '*microsporidian*' disease of the rabbit.

Pathological changes other than the above are found more con-

stantly in the Ammon's horn, especially in cases showing intense infiltrative lesions. For instance, a number of the nerve-cells may show degenerative changes characterized by the following appearances. The nucleus appears oedematous, the chromatin is fragmented, while the protoplasm shows more or less advanced tigrolysis. The protoplasm also contains vacuoles and the cellular membrane is denticulated. In some cells, also, of the Ammon's horn, the nucleus, and sometimes too the protoplasm, becomes oxyphilic. The fusiform cells which are found in the upper part of the row of large ganglion cells show intense nuclear degeneration; the karyoplasm is condensed in a 'block' and stains red with Mann's stain. When a preparation so stained was decolorized gradually, and examined after each stage of the process until these degenerated nuclei became a pale rose colour, it was found that they had no definite structure, but consisted of a round mass of homogeneous condensed chromatin much smaller in size than the unchanged nuclei. This type of nuclear degeneration has also been met with in the mesencephalon (Pl. III, Fig. 4).

We have already referred above to the fact that oxyphilic corpuscles which may or may not be surrounded by halos are found in the nucleus of some of the cells of the Ammon's horn. They can be distinguished from the nucleolus by their different staining reactions (Pl. I, Fig. 2). These intranuclear corpuscles—the specific 'inclusions' of the German workers—may be single or in twos or threes, varying in size. Sometimes they may be at the limits of visibility, or they may be as large as 2μ or 3μ. These corpuscles occur elsewhere. They may be found in the cytoplasm of the cell and are possibly expelled *intra vitam*, but as it is possible for the nucleolus of a cell to be dislodged by the microtome knife, the same factor might carry the intranuclear corpuscle into the cytoplasm.

Our opinion is that the intranuclear 'inclusion' in Borna disease is possibly a reaction of the karyoplasm against the pathological agent which penetrates the interior of the nucleus. Possibly the chromatin masses around the infective virus elements. This is suggested by the staining reactions, since the condensed mass of chromatin which forms the corpuscle undergoes degeneration from the centre towards the periphery (the centre appears oxyphilic and the periphery basiphilic in certain corpuscles).

Around the lateral ventricles well-marked infiltrations are found, these being in some cases very intense. The choroid plexus is also infiltrated. The epithelium of the ventricle and of the ependyma is unchanged.

Occasionally in the parenchyma in the region of the ventricle, or even in the cerebral cortex itself in the superficial areas, karyokinetic figures may be seen. Probably this karyokinesis is in mobilized cells of the vascular endothelium which have penetrated into the nerve substance. In certain preparations we have seen 2, 3, or even 4 karyokinetic figures. The mitosis sometimes undergoes oxyphilic degeneration.

There are lesions affecting the area above and below the ependyma and also the surrounding zone: a mobilization of the neuroglial elements takes place, while at the same time lymphocytic elements are found in the immediate proximity of the neuron. Up to a certain point this phenomenon is comparable with the 'satellitism' described by Metchnikoff in senility. Certain of the nerve-cells are surrounded on all sides by 'satellite' cells and cells of infiltration, which occasionally penetrate the interior of the cell. Six, eight, and even ten neurons may be seen 'besieged' in one microscopic field. The cells which come in immediate contact with the nerve-cell push in the cellular membrane and form 'cups' in the periphery of the cytoplasm, giving the nerve-cell a denticulated border. This phenomenon is more commonly met with in sections from rabbits dying within the first 20 days after inoculation. The intensity of 'satellitism', is in inverse proportion to the meningeal and perivascular lesions. When the meningitis and perivascular cuffing are at a minimum 'satellitism' may represent the only departure from the normal discovered in the brain.

From the examination of a large number of preparations we are led to believe that 'satellitism' is a stage which may either disappear during the evolution of the disease, resulting in a quasi-normal state, or become intensified and be followed by neuronophagia. We have found neuronophagia present in sections of brain showing marked 'satellitism' (Fig. 17). Our conception of the various stages of the struggle against the virus is as follows: When the nervous system is invaded by the virus the neuroglial elements and mononuclear lymphocytes are attracted to the parasitized neuron. If the neuron succeeds in freeing itself from the virus, the local reaction ceases at this stage, and the resorption of the satellite elements follows; but if the neuron dies in the struggle against the virus after undergoing intense degeneration, it is invaded by the satellite cells and rare polymorphonuclear leucocytes; the process has now reached the stage of neuronophagia. When the struggle between the neuron and the virus terminates without neuronophagia taking place the animal survives until the meningeal lesions, perivascular infiltrations, and infiltration of the Ammon's horn become incompatible with life. In this case death takes place at a later stage, i.e. in from 25 to 50 days.

These conclusions as to the evolution of the morbid process in the brain, formed from observations on the character and position of the lesions in a large number of rabbits dying early or late after inoculation, have received further support from the study of the histogenesis of the alterations in the central nervous sytem of six rabbits killed at regular intervals after inoculation, i.e. on the 5th, 10th, 15th, 20th, and 27th day, and of one which died on the 31st day. In these animals also, the microscopical examination of sections of various parts of the nervous system showed that the first modification in the central nervous sytem is the mobilization and proliferation of the neuroglial cells around the neuron—'satellitism', this being more marked in the pons and the medulla oblongata. In the process of 'satellitism' one finds not only neuroglial cells but also mononuclear

cells taking part. Later, infiltration of the *Cornu Ammonis*, the meninges, and the vascular tissues takes place. In the subjects of our experiments the latter process began to appear towards the 15th or 20th day after the inoculation.

The presence of polymorphonuclear cells is quite exceptional no matter at what stage of the infection or from what site one examines sections of the central nervous system; the infiltrative lesions are constituted from the beginning by mononuclear cells.

There are notable differences between the development of the encephalitis of Borna disease in the rabbit and chronic herpetic encephalitis produced experimentally in the same species. In the brain infected by herpes an acute stage is observed in which polymorphonuclear leucocytes take part, and are found in large numbers in the perivascular 'cuffing' as well as in the nodular lesions at the base of the brain in the region of the hippocampus. If the animal survives this acute stage and recovers what have been termed by Levaditi and Nicolau (1922) 'lésions d'immunité' may be found. These are small nodular or diffuse areas of parenchymatous infiltration situated in the hippocampus (the 'zone élective') and are produced by mononuclear cells that have replaced the polymorphonuclear leucocytes with which the inflammatory process commenced. In those rabbits which just fail to resist the disease and die in 20 to 30 days, the lesions are more intense, but, as in the case of the 'lésions d'immunité', the infiltration consists of mononuclear cells which have taken the place of the polymorphonuclear leucocytes that predominated in the acute stage of the inflammatory process. In the case of the infection of the brain of the rabbit with the virus of Borna disease, polymorphonuclear leucocytes do not play a part in the early inflammatory process; during this early stage, one observes only 'satellitism' of the neuron, while the infiltrative lesions are produced by mononuclear cells alone from the beginning of the process until the final stage.

(b) *The Mid-brain and Medulla Oblongata.* In the mesencephalon and the medulla oblongata similar infiltrative and degenerative lesions occur. 'Cuffing' of the vessels is frequently observed. Certain of the nerve-cells appear to be in a state of advanced tigrolysis (Nissl's granules have disappeared). Degeneration both of the nucleus and the cytoplasm is frequently found. Certain of the cells appear to have their protoplasm split up, the nucleus being peripheral, swollen, and completely degenerated (Pl. I, fig. 1). Occasionally typical neuronophagia is encountered. In these regions also we have demonstrated the presence of the intranuclear corpuscles of Joest-Degen. The cells which contain them generally preserve otherwise their normal aspect; the nuclear membrane is intact, while the protoplasm is structurally unchanged and stains normally. The converse is also true; we have never found the corpuscles of Joest-Degen in cells in advanced stages of degeneration or disintegration. Negri bodies in rabies are also only found in nerve-cells which are otherwise normal. In the mesencephalon, as mentioned above, one meets most

frequently with 'satellitism' of the neuron, which in certain cases goes so far as to constitute true neuronophagia (Fig. 18). In the mesencephalon also we may find small islands of mononuclear cells in the parenchyma without any relation to the vessels. In certain cases we found neuroglial cells (granulo-adipose cells) showing nuclear oxychromasia and occasionally small oxyphilic corpuscles within the nucleus, similar to the so-called 'encephalitis neurocorpuscles' of herpes described by Levaditi, Harvier, and Nicolau (1922).

(c) *Cerebellum.* In the case described the lesions in the cerebellum were much more intense than the average, since usually the alterations consisted only of slight meningitis and perivascular infiltration accompanied by occasional 'satellitism' of the cells of Purkinje. In the septum there was a marked infiltration with mononuclear cells. Massive perivascular 'cuffing' was present, especially in the white substance between the convolutions. In the vessels themselves, which were gorged with blood, an excessive number of mononuclear cells were found. There was an intense infiltration in the granular layer, which in some cases was completely destroyed and replaced by areas formed exclusively of mononuclear cells. Here and there in the islands of lymphocytes 'basket' cells with a pale-staining degenerated protoplasm were found, their border appearing irregular. Several of the cells of Purkinje appeared to be hyperchromatic, and their nucleus was in some cases eccentric and stained by acid stains. In others the nucleus was not separated from the rest of the protoplasm since the remains of the nuclear membrane appeared to have disappeared. Other Purkinje cells were degenerated and appeared as cell shadows. In other parts the karyoplasm of certain of these cells was condensed around the nucleolus. Satellitism of the basket cells and the cells of Purkinje was noticeable, but true neuronophagia was not seen. The lesions were confined to certain areas; other parts of the cerebellum were perfectly normal.

In a case where the inoculation of the virus was made by the intratesticular route the lesions in the cord were especially well marked, and in the cerebellum the satellitism of the cells of Purkinje was occasionally so advanced as to constitute almost a true neuronophagia.

(d) *The Spinal Cord.* Generally it may be stated that the intensity of the lesions found in the brain or in the spinal cord corresponded with the intensity of the symptoms observed during life. In the rabbits we have examined pathological changes were always present in the cord whether symptoms of affection of this part of the central nervous system were present or not, but these were much more intense when the symptoms produced by affection of that region dominated the cerebral symptoms.

As in the case of the brain, the meninges of the cord are not as a rule greatly affected. Only in isolated cases was a severe meningitis found.

The anterior and posterior septa may be more or less infiltrated with mononuclear cells. Perivascular 'cuffing' is seen both in the

grey and white matter of the cord. In the anterior and posterior horns infiltrations with mononuclear cells may be seen.

The most intense infiltrations are found in the posterior horn, while in the anterior horn degenerative lesions of the neuron are more common. The process of degeneration in the nerve-cells is the same as that in other regions of the central nervous system: tigrolysis occurs, nuclear oxychromasia exists, while the whole cell shows a marked hyperchromasia. Vacuolization of the cytoplasm, a degenerative process, may also be seen (Pl. II, Fig. 1), while in rare cases when the lesions as a whole have been exceptionally intense, occasional neuronophagia was recorded (Fig. 19). The phenomenon of 'satellitism' appears to be more commonly met with in the cord than in the brain itself. The intranuclear corpuscles of Joest-Degen found in the cord have generally been in the nerve-cells of the anterior horn (Pl. I. Fig. 5; Pl. III, Fig. 2).

Here and there small islands of lymphocytes may be found infiltrating both the white and the grey substance, these islands being unconnected with vessels. Frequently it has been observed that the zone of Lissauer is the site of a well-marked mononuclear infiltration.

The lesions found in the spinal cord are comparable with those found in poliomyelitis. The neuronophagia so characteristic in the cord of monkeys infected with the virus of the latter disease (to which rabbits are generally considered not to be susceptible) is also present in the cord of rabbits infected with the virus of Borna disease, but to a less degree.

(3) *Lesions in the Peripheral Nervous System.*

(a) *The Posterior Nerve-roots* arising from the cells in the zone of Lissauer have infiltrative lesions which vary in intensity. This zone, as has been stated above, is generally infiltrated with mononuclear cells. The infiltration takes place between the nerve filaments, and consists of a chain of lymphocytes. In some cases only traces of this infiltration can be seen, while in other cases massive perivascular infiltration may occur (Fig. 20). The interstitial infiltration in the anterior nerve-roots is very discrete or absent.

This process of radiculitis has not been mentioned by other workers who have studied the disease in animals infected experimentally; nor has the process of infiltration of the nerve-roots been described in the spontaneous disease.

(b) *The Spinal Ganglia.* The most intense lesions in the peripheral nervous system have been found constantly in the spinal ganglia. The process of infiltration in the posterior nerve-roots becomes more intense as they enter the ganglion, and between the nerve-fibres which pass through the substance of the ganglion a well-marked mononuclear infiltration is seen. In the rest of the ganglion the lesions as a rule are very intense.

The alterations in the ganglion and the various elements taking part in the infiltrative and degenerative processes at this site are always the same, no matter from what individual case or from what region

of the cord (cervical, thoracic, or lumbar) the ganglion is taken (Figs. 21, 22). The changes are as great and the lesions of the same importance when the ganglion originates from a case showing alterations in the cord which are scarcely discernible as from a case where such changes are very pronounced.

The capsule of the ganglion shows neither infiltration nor degeneration. In the interior of the ganglion mononuclear interstitial infiltration is abundant. The small intraganglionic vessels show perivascular 'cuffing'. The infiltrating mononuclear elements are found disseminated between the nerve-cells or massed together forming actual nodules comparable with those described by Van Gehuchten and Nelis (1900) in rabies (Fig. 23). The mononuclear cells may be grouped together in small islands between the nerve fasciculi which traverse the ganglion. The ganglion cells themselves appear to be 'choked' by the infiltrative process in some microscopic fields. In certain parts these cells undergo profound changes: the nucleus becomes oxyphilic, the protoplasm loses its granular nature, assuming a homogeneous appearance, and becomes slightly oxyphilic when stained with toluidin blue and eosin. We have found that the changes in the cells of the ganglion are more marked than in any other region of the nervous system; and the intranuclear corpuscles are larger and in greater number here than in any other site. In some microscopic fields the nucleus of every cell may contain one or two corpuscles of Joest-Degen surrounded by a halo.

The most important and frequent type of lesion in the ganglion, however, is neuronophagia. Lymphocytes, plasma cells, and large mononuclears penetrate the peripheral zone of the neuron. One often finds a clear zone in the protoplasm around these infiltrating cells suggestive of the action of a proteolytic ferment liberated by the invading cells. Later the mass of detritus of the neuron is removed by the macrophages aided by occasional polymorphonuclear leucocytes. The number of infiltrating cells increases, the whole body of the nerve-cell being invaded, and finally, in place of the neuron, one finds nothing but a nodule formed by mononuclear cells (Figs. 23, 24, 25, and 26). The most intense lesions of both an infiltrative and degenerative character are found in the peripheral zone of the ganglion; this point will be discussed again later.

(c) *The Peripheral Nerves.* In the peripheral nerves infiltrative lesions are also found. A detailed study has been made of lesions found in the sciatic and brachial nerves. The technique employed in carrying out this research was as follows:

All the rabbits of which the sciatic and brachial nerves were sectioned for histological examination had been inoculated intracerebrally with the virus of Borna disease. We removed the terminal part of the cord (sacral) with the roots of the sciatic nerve and their various ganglia together with a portion of the peripheral parts of the nerves. This whole was fixed in Duboscq-Brasil-Bouin fluid. Longitudinal sections were made after the manner figured (Fig. No. 27).

We have found lesions in all cases examined, these being more

intense towards the origin of the nerve and becoming less intense towards its termination. The alterations consist of interstitial or perivascular infiltrations with mononuclear cells. The nerve-sheath is, as a rule, unaffected. In certain cases the infiltrations appear to 'dissect' the nerve filaments (Fig. 28). The whole process constitutes a descending neuritis produced by the virus propagating centrifugally.[1]

Recently G. Marinesco and S. Draganesco (1927) published their observations on the pathogenic process in herpes zoster. A complete clinical report is given of cases in which the localization of the lesions in the nervous system suggested to the authors that the infection commenced by an ascending neuritis followed by a ganglio-radiculitis and myelitis. Wohlwill (1924), Levaditi (1926), Pette (1924), Foerster (1924), and others advanced similar hypotheses as to the centripetal propagation of the infection. In support of their theory as to virus ascending from the peripheral nerves, Marinesco and Draganesco refer to the lesions in the corresponding ganglia: 'In the ganglion the most intense lesions were in the peripheral zone . . . this topography of the inflammation explains the spread of the infection by the pericapsular lymph vessels, to the interior of the ganglion.'

However, from a comparison of the description of the lesions produced by the downward extension of the virus in rabbits infected with the virus of Borna disease, and those in herpes zoster, it will be seen that the histological pictures are identical. We have found lesions in the peripheral nerve not only close to the ganglion as described by Marinesco in zoster, but also in the terminal filaments farthest removed from the ganglion, showing that the virus in our experiments diffused by centrifugal propagation. These facts allow us to assume that the topography of the lesions is not a criterion by which to judge the portal of entry of the virus with a sufficient degree of accuracy. Comparable lesions can be produced in the central and peripheral nervous system both by infection intracerebrally or by inoculation of the virus into the sciatic, i.e. no matter whether the infection is ascending or descending.

This example of lesions being produced in the peripheral nervous system after introduction of the virus into the central nervous system (brain), suggests the possibility of infection being central in origin in the case also of herpes zoster. A similar pathogenic process is not excluded in recurrent herpes, peripheral forms of epidemic encephalitis, and perhaps also in certain cases of sciatica.

(4) *Summary and Discussion.*

The inoculation of the virus of enzootic encephalo-myelitis intracerebrally into rabbits produces changes in the nervous system, which are those of a meningo-encephalo-myelitis, a ganglio-radiculitis, and a peripheral interstitial neuritis.

The lesions in the central nervous system as well as in the spinal

[1] We have been able to demonstrate the presence of virus in the peripheral nerves by inoculation of their emulsions into the brain of rabbits (see p. 45).

ganglia are both infiltrative and degenerative. The meningitis and the perivascular and parenchymatous infiltrations are produced by mononuclear cells.

Pathological 'satellitism' of the neuron is most pronounced in the mesencephalon, medulla oblongata, and spinal ganglia, but may be found also in other regions of the cord and brain. It may in some cases be so advanced as to constitute true neuronophagia. The latter phenomenon is most common in the paravertebral ganglia.

The intranuclear corpuscles of Joest-Degen, considered by the present writers to be evidence of an attempt at defence by the nerve-cell, and referred to as specific 'inclusions' by other workers, may be found in the various regions of the brain, cord, and spinal ganglia. They are almost constantly present in the large ganglion cells of the *Cornu Ammonis* and the nerve-cells in the spinal ganglia. In our opinion the cell which reacts against the presence of the virus by the formation of intranuclear corpuscles has formed a barrier to the extension of the destructive action of the virus in 'blocking' the infective elements within a condensation of its chromatin. It is feasible to conceive that this process removes the virus and renders it inoffensive: for this reason the cell maintains its integrity. In those cases where the cell becomes degenerated or neuronophagia takes place, one may suppose that the nucleus has been incapable of surrounding the infective particles by condensation of its chromatin and thus limiting the extension of the activity of the virus. This failure to form intranuclear corpuscles may be due to the quality of the virus (virulence), the quantity of the virus, or the deficiency in the normal resisting power of the neuron, the result being that the virus multiplies and ultimately destroys the cell. One must recall that the figure described on p. 56 supports this view as to the method of production of the corpuscles of Joest-Degen.

The infiltrations in the nerves are interstitial in character and are produced by the invading mononuclear cells arranging themselves in chains between the nerve filaments. Perivascular 'cuffing' also occurs.

A peripheral interstitial neuritis occurs in Borna disease after the introduction of the virus into the brain, and the authors, without excluding the possibility of ascending infections, have suggested that herpes zoster,[1] recurrent herpes, the peripheral forms of epidemic encephalitis, and perhaps also certain forms of sciatica may be the secondary manifestations of a disease, the original focus of which is in the central nervous system.

The hypothesis has already been advanced (see p. 38) that in cases of infections with these viruses central infection of the brain takes place; but the central nervous system, being able to resist the action of the virus more efficaciously, shows no manifest disturbances, while the peripheral nerves, poor in methods of defence, do not rid themselves of the infecting elements which proliferate and produce lesions.

[1] The work of Head and Campbell (1900) on the pathology of herpes zoster also suggests that zona is a secondary peripheral manifestation of a disease originating in the central nervous system.

B. *The Guinea-pig.*

Macroscopic Examination. The brain and spinal cord appeared congested. No other organ showed pathological changes except the stomach, in which occasionally the non-specific lenticular haemorrhagic areas, similar to those described in the rabbit, were found.

Microscopic Examination. Lesions were found in the central and peripheral nervous sytem and in the kidney. The alterations in the stomach wall, when they existed, were comparable with those found in the rabbit, namely, autodigestion of the mucosa with small localized haemorrhage. As in the case of the rabbit, the kidneys showed marked congestion, but no actual nephritis was recorded.

Central Nervous System. The lesions found in the brain were similar to those in the rabbit, except that their intensity was less. The intranuclear corpuscles of Joest-Degen were usually found in the *Cornu Ammonis* and elsewhere (Pl. I, Fig. 4). Infiltrative and degenerative lesions characteristic of the disease were found (Pl. III, Fig. 3).

The lesions in the mesencephalon, cerebellum, and spinal cord were as in the rabbit. The intranuclear corpuscles of Joest-Degen were frequently present in the anterior horn of the spinal cord.

Peripheral Nervous System. Infiltrative processes with mononuclear elements were found in the nerve-roots, but they were not so marked as in the rabbit. The lesions in the spinal ganglia were similar in nature to those described in the rabbit, but were not so acute. The lesions in the peripheral nerves were more intense in that portion nearest the ganglia.

C. *The Rat and Mouse.*

The organs, except the nervous system and the kidney, were macroscopically and microscopically normal. In the brain and spinal cord the lesions found were similar to those in the rabbit and the guinea-pig. The intranuclear corpuscles of Joest-Degen were found in the brain of rats and mice. Infiltrative lesions of a discrete nature were also found in the sciatic nerve of the rat.

D. *The Monkey* (Macacus rhesus).

Our description of the lesions found in the monkey is made from a study of sections from different parts of the nervous system of Monkey M. 1 (*Macacus rhesus*). The protocol of the experiment in which this animal was infected is given on p. 34. Similar lesions were found in Monkey M. 3, although the clinical picture in the case of the latter monkey was different from that of M. 1.

The macroscopical and microscopical examination of the spleen, liver, pancreas, lung, myocardium, testicle, ovary, parotid gland, and the mesenteric and inguinal lymph glands did not reveal any pathological changes in these organs; the kidneys were hyperaemic. In the adrenal gland there was slight infiltration with lymphocytes in the medullary zone, the lymphocytes being disseminated in the

parenchyma or grouped together in small islands. The brain appeared to be normal by naked-eye examination.

(1) *Lesions in the Central Nervous System.*

(a) *The Brain. Frontal Lobe.* Meningitis of a mild character was present in some areas, becoming intensified near those vessels in which slight perivascular infiltrations were observed. The pathological process in the meninges might be described as an 'irritation' rather than a true meningitis, while the perivascular 'cuffing' consisted of three or four layers of cells only in the walls of the vessels in contact with the brain; in the rest of the vessels the process of infiltration hardly existed. Certain of the small vessels penetrating the cerebral parenchyma from the meninges were surrounded by characteristic 'cuffing'. There was infiltration with mononuclear cells in the septum. The lesions in the meninges, the 'cuffing' (Fig. 30), and the infiltration of the septum, were produced by lymphocytes, plasma cells, and macrophages exclusively. In the parenchyma, and especially in the white matter, extensive 'cuffing' of the vessels could be seen, consisting of ten to twenty layers of infiltrative cells. Some of the pyramidal cells appeared to be degenerated. Intense satellitism of the neuron was present in some instances, and in certain of the cells oxyphilic corpuscles surrounded by a halo of the type described by Joest and Degen were found (Pl. III, Fig. 1). The karyoplasm was rarefied in the greater number of the cells containing 'inclusions', suggesting that the degenerated chromatin was condensed in the corpuscles. In the deeper part of the brain, both satellitism and the intranuclear corpuscles of Joest-Degen were less frequent than in the peripheral zone.

Parietal Lobe. The meninges were infiltrated with mononuclear cells (Fig. 29) which formed plaques in certain regions. There was discrete infiltration of the septum. In the brain substance, perivascular infiltrations, consisting of many layers of cells, were found forming small nodules: that these were perivascular was evidenced by the presence of a small vessel in the centre of the nodule (Fig. 31). No neuronophagia was recorded in this region of the brain, although the acute 'satellitism' of the neuron sometimes suggested the phenomenon. Occasional nerve-cells in a state of degeneration had eccentric nuclei and their protoplasm was undergoing tigrolysis. No actual parenchymatous infiltration could be seen, but rare mononuclear cells were dispersed in the parenchyma. A large number of nerve-cells in this region contained large oxyphilic corpuscles surrounded by halos within the nuclei.

Occipital Lobe. Meningitis was rarely observed in this region of the brain. Certain of the meningeal vessels had several layers of mononuclear cells on their walls in contact with the brain. In the septum, infiltration was not well marked, although several venules were surrounded by 'cuffing'. Rich perivascular infiltrations were found in the parenchyma, more especially near the large pyramidal cells. Pathological 'satellitism' of the neuron by neuroglial elements and

occasional lymphocytes was not uncommon in the occipital lobe: these satellite cells sometimes produced marked depressions in the protoplasm of the host cell. The intranuclear corpuscles were of very much smaller dimensions and were observed less frequently than in the parietal lobe.

Hippocampus. The lesions in the meninges were similar to those found in the occipital lobe. The perivascular 'cuffing' in the parenchyma was poor in elements. There was a slight infiltration with mononuclear cells between the large and small pyramidal cells. The number of 'inclusions' was greater in this region than in the occipital lobe.

Cornu Ammonis. In this region of the brain the 'cuffing' around the vessels was so extensive as to suggest a nodule or pseudo-gumma: the presence of a small vessel in the centre of the nodule was discerned with difficulty. In addition, there were small groups of mononuclear cells bearing no relation to vessels. In certain cases 'satellitism' was so advanced that it could almost be described as neuronophagia. The large ganglion cells preserved their normal structure and nearly all contained within their nucleus well-marked corpuscles of the Joest-Degen type.

Basal Ganglia. Perivascular 'cuffing' was observed. In some of the degenerated nerve-cells the nucleus could not be differentiated from the rest of the protoplasm. Pathological 'satellitism' of the nerve-cells was intense. Intranuclear corpuscles were found on occasion, not only in the nucleus but even in the cytoplasm. Small islets of mononuclear cells were also found.

Pons. The process of perivascular infiltration was abundantly present in this region.

Many of the nerve-cells were in an advanced stage of degeneration, while the nuclei were swollen and unrecognizable as such. The protoplasm of the cells was in a state of tigrolysis and its contour was broken in many places by splitting. The cells containing intranuclear corpuscles were otherwise morphologically normal. No areas of infiltration were seen.

(b) *Cerebellum.* There was slight infiltration of the meninges and septum with mononuclear cells: the parenchyma was not infiltrated. The layer of the small granular cells had a normal aspect. Certain of the cells of Purkinje were degenerated. In some of the latter cells oxyphilic corpuscles with a characteristic halo were recorded.

(c) *Medulla Oblongata.* Meningeal changes were slight. Vessels were normal without 'cuffing' and the parenchyma was not infiltrated. Occasional neurons showed evidence of commencing degeneration, and in certain of these large oxyphilic corpuscles were present.

(d) *Spinal Cord. Cervical Region.* The meninges were normal and no 'cuffing' was present in them or in the grey or white matter. A slight diffuse infiltration was observed. In the anterior horns non-degenerated neurons had intranuclear corpuscles (Joest-Degen type). In certain regions satellitism was a marked feature. The zone of the cells of Lissauer was infiltrated with lymphocytes. The *posterior*

nerve-roots showed interstitial infiltration and sometimes even 'cuffing' in the vessels. The corresponding *spinal ganglia* showed intense interstitial infiltrations: only rare polymorphonuclear leucocytes were found. The protoplasm of certain of the cells in the ganglia had undergone tigrolysis, and in some cases also had become oxyphilic; the nucleus of these affected cells was eccentric. Neuronophagia was frequently seen and nodules of mononuclear cells comparable with those found in rabies were not uncommon. Large intranuclear corpuscles were found in nerve-cells of the ganglia which otherwise preserved their morphological integrity (Fig. 35).

Thoracic Region. No meningitis was present, nor was there any evidence of 'cuffing'. There was slight infiltration of the posterior horns and a concomitant degeneration of certain nerve-cells of the anterior horns. In the latter region also rare intranuclear corpuscles were found (Pl. II, Fig. 2). The posterior nerve-roots showed interstitial infiltration and 'cuffing' around the vessels.

Lumbar Region. No perivascular infiltrations and no meningitis could be seen. There was a slight diffuse parenchymatous infiltration with lymphocytes. Satellitism of the neuron was present, both in the anterior and the posterior horns. There were well-marked lesions in the lateral horns: the protoplasm was markedly oxyphilic and the nuclei of the cells were eccentric. Sometimes the satellite cells penetrated the protoplasm of the degenerated neurons and constituted almost true neuronophagia. Certain small nerve-cells of the anterior horn showed neuronophagia. Intranuclear corpuscles were rare. Interstitial infiltrations and 'cuffings' were seen in the posterior nerve-roots (Fig. 34).

In the spinal ganglia the lesions were of a similar nature to those encountered in the upper regions of the cord, but were more intense (Fig. 36). Neuronophagia was a constant feature. The nuclei of cells containing intranuclear corpuscles of the Joest-Degen type showed a rarefication of the karyoplasm, but no other morphological changes were present in such cells (Pl. III, Fig. 5).

Sacro-caudal Region. In the large nerve fasciculi rich infiltration with mononuclear cells could be seen, together with marked 'cuffing' round the vessels (Fig. 33).

In the posterior roots of the nerves the interstitial infiltration and 'cuffing' round the vessels was rich.

The spinal ganglia showed similar lesions to those recorded in the lumbar region. The capsules of such ganglia were unaffected, and the lesions were marked in the periphery of the ganglion. In the nerves, after their exit from the ganglion, lesions similar to those seen in the nerve-roots were found. Massive infiltration with mononuclear cells was seen between the fasciculi of the nerves (epineurium), in the connective tissue around the nerve (perineurium), and even in the endoneurium. 'Cuffing' of the vessels was also seen.

(*e*) *Sciatic Nerve.* A study of transverse sections from the sciatic nerve after its exit from the greater sciatic foramen showed that there was a diffuse infiltration between the nerve fasciculi; this was

generally more pronounced in the interior of the fasciculi. In the thickness of the nerve an intense perivascular infiltration was seen (Figs. 38 and 39). Often the connective tissue of the sheath was unchanged, while intense infiltrative lesions were seen in the thickness of the nerve.

Examination of longitudinal sections made from the sciatic nerve half-way between the greater sciatic foramen and the popliteal region showed that interstitial infiltration with mononuclears existed along with massive perivascular 'cuffing', while the nerve-sheath itself appeared perfectly normal.

(f) *Brachial Nerve.* The alterations seen in this nerve were analogous to those described in the sciatic nerve; even in the lower third of the fore limb intense lesions were found (Fig. 37).

The infiltrative lesions of the nerve-roots and peripheral parts of the nerves, as well as the alterations in the paravertebral ganglia, show that in the monkey, as in the rabbit infected intracerebrally with the virus, the infective agent travels from the central nervous system to the periphery along the nerves, producing the lesions described above, which constitute a *ganglio-radiculatis* and *descending peripheral interstitial neuritis.*

(2) *Summary.*

In the *brain* there existed a mild meningitis with perivascular infiltrations and diffuse parenchymatous infiltrations, which sometimes formed actual nodules; massive perivascular infiltration was also seen in the parenchyma; there was degeneration of nerve-cells and satellitism. Intranuclear corpuscles of the type described by Joest and Degen were more easily found and more numerous than in the rabbit.

In the *cerebellum* there was found a slight meningeal reaction with perivascular 'cuffing', satellitism of the cells of Purkinje in certain areas, and rare oxyphilic corpuscles (Joest-Degen type) in the nuclei were recorded.

In the *spinal cord* no meningitis was recorded and there was an absence of 'cuffing' in the vessels of the coverings of the cord. In the posterior horn, a diffuse infiltration was observed, this being more marked in the lumbar region. In this region of the cord also a similar infiltration was seen in the anterior and lateral horns. In the anterior horns degeneration in the nerve-cells was recorded, and sometimes neuronophagia.

In the *peripheral nervous system* an infiltrative radiculatis existed.

The *spinal ganglia* were intensely affected, showing lesions both infiltrative and degenerative; neuronophagia was a common feature.

A peripheral neuritis (*sciatic and brachial nerves*) was present, consisting of interstitial and perivascular infiltration.

The topography of the lesions as a whole shows that the virus introduced into the brain produces lesions locally, spreads to the rest of the central nervous system, and finally travels down the peripheral nerves. The cells in the lesions are almost exclusively mononuclears.

11. IMMUNITY

All authors agree that in the horse an attack of enzootic encephalomyelitis contracted spontaneously does not render this animal immune to a second attack; with regard to the disease in cattle and sheep, no precise records are available as to this point. The rabbit appears to behave differently. Zwick (1926) and his collaborators observed that in one case a rabbit which had been infected experimentally and had shown symptoms typical of the disease ultimately recovered and resisted a second intracerebral inoculation. The same authors succeeded, although not constantly, in producing a solid immunity by repeated inoculation of virus either subcutaneously or intravenously. They showed likewise that the introduction of a large quantity of virus intraperitoneally may render rabbits refractory to subsequent infection. Ernst and Hahn (1926) have shown that the inoculation of virus intracerebrally into rabbits does not lead to the development of the disease, if, during the period of incubation, such animals receive repeated inoculations of virulent material into the veins.

Zwick and his collaborators (1926), in the few experiments which they record in attempts to demonstrate antibodies in the serum of immunized animals by neutralization of virus *in vitro*, did not obtain very conclusive results.

In passaging the strains of virus with which we have been working, viz. a strain originating from horses (Zwick) and a strain originating from sheep (Miessner), we have never found any healthy uninoculated rabbit refractory to infection.

I. Attempts at Conferring Immunity to Rabbits by Inoculations of Non-attenuated Virus.

A. *Intravenous Inoculatian.*

(1) Four rabbits were inoculated intravenously with 1·5 c.cms. of a virulent centrifugalized emulsion, which, when inoculated into the brain of a rabbit, produced the disease and death at the end of 39 days. The rabbits inoculated intravenously did not show any symptoms and gained weight. Between 91 and 206 days later they were inoculated intracerebrally with fresh passage virus. Table X. below shows that a single intravenous inoculation of virulent emulsion does not produce immunity in rabbits.

(2) Another rabbit, No. 69, weight 1,980 gms., received three inoculations of virulent material into the veins.

16.5.27. First intravenous inoculation of 3 c.cms. of centrifugalized emulsion.
21.5.27. Animal normal. Weight 1,780 gms.
29.5.27. Animal normal. Weight 1,640 gms.
30.5.27. Second intravenous inoculation of 5 c.cms. of virulent emulsion.
8.6.27. Animal normal. Weight 1,600 gms.
13.6.27. Third intravenous inoculation of 1·5 c.cms. of virulent emulsion.
14.6.27. Animal normal. Weight 1,680 gms.
22.6.27. Animal normal. Weight 1,670 gms.

TABLE X.

No. of rabbit.	*Weight.*	*Result of intravenous inoculation.*	*No. of days after the intravenous inoculation that the rabbit received the intracerebral inoculation.*	*Weight at time of intracerebral inoculation.*	*Result.*	*Controls of intracerebral inoculation.*
294	1,720 gms.	Nil	91 days	2,500 gms.	Dead 44th day	Rabbit 91A dead 27th day.
288	2,080 ,,	,,	97 ,,	2,440 ,,	Dead 36th ,,	Rabbit 775 dead 13th day.
296	1,820 ,,	,,	97 ,,	2,900 ,,	Dead 33rd ,,	With passage positive.
297	1,920 ,,	,,	206 ,,	2,800 ,,	Dead 39th ,,	Rabbit 25A dead 48th day.

On 23.6.27 the rabbit received a test inoculation of virulent material at the same time as a control. The control died after 34 days with symptoms and lesions characteristic of the disease in the central nervous system. The rabbit prepared by intravenous inoculations behaved as recorded below.

30.6.27. Animal normal. Weight 1,750 gms.
7.7.27. Animal normal. Weight 1,950 gms.
10.7.27. Animal normal. Weight 1,680 gms.
14.7.27. Commencement of the disease. Weight 1,580 gms.
23.7.27. Typical symptoms of the disease. Weight 1,400 gms.
26.7.27. Animal died during the day. Weight 1,230 gms.

Microscopic examination of the brain and spinal cord revealed the presence of characteristic lesions.

In this case three intravenous inoculations did not lead to immunity to cerebral infection.

That four intravenous injections of virulent material may, however, produce a solid immunity is shown by the experiment recorded on p. 75, Rabbit 67.

That the intracerebral inoculation of a filtrate (Mandler filter) of virulent emulsion does not lead to the development of immunity may be concluded from the experiments mentioned on p. 13.

The effect of introducing a thick emulsion of virulent brain into the trachea of rabbits was tested, but it did not produce the disease nor immunity in animals so treated. 0·5 c.cm. of a virulent emulsion of brain was introduced into the trachea of three animals. As they did not present any morbid symptoms for 174 days they were inoculated intracerebrally with a virulent emulsion. They all died from enzootic encephalo-myelitis after 37, 27, and 40 days respectively. (See p. 33.)

Rabbits which survive corneal or intratesticular inoculations of virulent material may become refractory to the disease as tested subsequently by intracerebral inoculation.

B. *Corneal Inoculation.*

The experiment recorded on p. 31 (Rabbit 81s) shows that infection by the corneal route can immunize the rabbit against a subsequent inoculation by the cerebral route.

C. *Intratesticular Inoculation.*

Some measure of immunity may also follow intratesticular inoculation. Two rabbits (273 and 289) were inoculated into the right testicle with 1 c.cm. of a virulent cerebral emulsion. One of these rabbits, No. 289, died after 105 days. Typical lesions were found in sections of the various parts of the central nervous system, and passage of its brain to a fresh rabbit gave a positive result.

The other rabbit, No. 273, survived, and when inoculated by the

intracerebral route 112 days later it proved to be immune. The control rabbit inoculated by the intracerebral route died on the 48th day.

II. Attempts at Conferring Immunity to Rabbits by Inoculation of Attenuated Virus.[1]

A. *Virus Inactivated by Ether.*

Roux[2] showed the attenuating action of ether on the virus of rabies. Later Remlinger (1919) used an ether-treated virus subcutaneously to produce an immunity in rabbits to intracerebral inoculation with fixed virus. Alvisatos (1922) and Hempt (1925) used ether-treated virus as a means of vaccinating man against rabies.

Marie and Mutermilch (1927) have shown that one can immunize rabbits against rabies by inoculating intrathecally virus treated with ether. We have tried to immunize rabbits against the virus of encephalo-myelitis, employing a similar technique, with the difference that the virus treated with ether was inoculated into the brain; the proof of the avirulence of the virus treated with ether and the technique employed has been described on p. 22.

The results obtained are given below.

Two rabbits, 220A and 217A, weighing 2,450 and 1,950 gms. respectively, received two intracerebral inoculations of 0·5 c.cm. of ether-treated virus at an interval of 5 days. Twenty-one days after the last inoculation they were inoculated intracerebrally along with a control rabbit, 14A, with fresh virus. Rabbit 217A died on the 42nd day, Rabbit 220A on the 17th day, and control Rabbit 14A on the 30th day after inoculation, and typical microscopic lesions were found in the central nervous system of all three.

Thus two successive intracerebral inoculations into rabbits of virus treated with ether did not produce immunity to subsequent intracerebral inoculation.

B. *Virus killed by Chloroform.*

The proof that virus treated with chloroform is inactivated has already been given on p. 21. In the experiment about to be described, rabbits were twice inoculated intracerebrally with virus treated with chloroform, the interval between the inoculations being 5 days. The animals were tested 3 weeks after the second inoculation, and since the experiments were made at the same time as the experiments with ether-treated virus, the control Rabbit 14A served for both.

The rabbits prepared with virus treated with chloroform succumbed

[1] The experiments in attempts to immunize with virus treated with ether, chloroform, or formalin recorded are preliminary and for orientation; it is possible that modifications in technique might change the results originally obtained. There are at present under experiment other series of animals, and the results obtained in these subsequent attempts will be recorded at some future date.

[2] Roux's unpublished observations.

to a subsequent intracerebral infection after 37 and 42 days respectively. Cultures made from these brains were negative, but lesions characteristic of infection with Borna disease were found on microscopic examination of sections of brain, cord, and spinal ganglia.

C. *Virus treated with Formol.*

On p. 22 it was shown that virus treated with 0·2 per cent. formalin for 18 hours at room temperature failed to infect rabbits by the intracerebral route.

The vaccine was prepared the day before use by subjecting fresh virulent brain material to the action of formalin in this concentration. A batch of ten rabbits was prepared by subcutaneous inoculation of this vaccine: the results of the experiment are recorded in Table XI. The results obtained in this experiment were not very satisfactory. Of the ten rabbits prepared by inoculation of formolized virus two died accidentally, five died following upon infection with the test dose given intracerebrally, and three proved to be immune to the test dose given intracerebrally.

D. *Virus Inactivated by Ultra-violet Light.*

An experiment on this subject is recorded on p. 20 in which a rabbit which survived after having received an inoculation of virus exposed for 5 minutes to the action of rays emitted by the mercury arc proved to be still susceptible to infection by the intracerebral inoculation of virulent material.

III. Search for Virucidal Antibodies in the Serum of Immunized Animals.

The serum of the immune rabbit, 223A (see p. 75), was taken and mixed with equal parts of a centrifugalized virulent cerebral emulsion; at the same time two further mixtures of the same cerebral emulsion were made, one with equal parts of normal rabbit serum and the other with physiological saline. The three mixtures were kept for 2 hours at 37° C. and were subsequently inoculated intracerebrally into rabbits.

The results were: (1) the rabbit inoculated with the mixture of virus and immune serum died on the 14th day after inoculation; its brain passaged to a fresh rabbit killed in 35 days; the lesions found in sections of the brain of both of these rabbits were those of experimental Borna disease.

(2) The control rabbits inoculated with the mixture of normal rabbit serum and virus died 39 and 34 days respectively after inoculation of Borna Disease, the lesions found on microscopical examination of sections of the nervous system being typical.

(3) The rabbits inoculated with the mixture of virus and physiological saline also succumbed to the inoculation.

TABLE XI.

No. of rabbit.	*1.9.27.* *1st inoculation of 5 c.cms. of formol-treated virus.*	*5.9.27.* *2nd inoculation of 5 c.cms. of formol-treated virus.*	*10.9.27.* *3rd inoculation of 3 c.cms. of formol-treated virus.*	*15.9.27.* *4th inoculation of 5 c.cms. of formol-treated virus.*	*20.9.27.* *5th inoculation of 5 c.cms. of formol-treated virus.*	*Intracerebral inoculation.*	*Results.*
	Weight in gms.	*Weight in gms.*	*Weight in gms.*	*Weight in gms.*	*Weight in gms.*		
15A	2,260	2,300	2,300	2,270	2,200	Brain	Died accidentally.
16A	1,920	1,900	2,100	2,040	1,750	,,	Survived.
17A	1,910	2,000	2,100	2,040	1,730	,,	Died accidentally.
18A	1,800	1,900	1,900	1,880	2,010	,,	Died 37th day.
19A	2,170	2,100	2,080	2,060	2,250	,,	Survived.
20A	2,150	2,100	2,150	2,080	2,250	,,	Died 36th day, after showing typical symptoms.
21A	2,590	2,450	2,380	2,350	2,300	,,	Died 27th day, after showing typical symptoms.
22A	1,290	1,250	1,260	1,260	1,290	,,	Died 27th day as above.
23A	1,590	1,600	1,750	1,900	1,880	,,	Survived.
24A	1,330	1,350	1,350	1,320	1,230	,,	Died 38th day with characteristic symptoms.
68A		Control not treated with formolized virus				,,	Died 28th day: typical symptoms and lesions.
69A		,,	,,	,,	,,	,,	Died 28th day: typical symptoms and lesions.

IV. Experiments on Cross Immunity between the Strain of Equine and that of Ovine Origin.

Our experiments have been carried out with two strains of enzootic encephalo-myelitis, the one originating from the horse (Zwick's strain), and the other from the sheep (Miessner's strain).

Experiment 1. Rabbit 223A. Weight 2,280 gms. A diluted emulsion of virulent brain (Zwick strain) which had been pulped and preserved in glycerine at room temperature for several weeks was inoculated into the brain on 22.10.26. The animal lost weight slightly, developed paresis of the hind quarters about the 20th day after the infection, but subsequently recovered, and on 15.12.26, 54 days after the inoculation, it appeared perfectly normal and had a weight of 2,400 gms. On this date it was inoculated intracerebrally with fresh passage virus (Zwick strain) and survived, while the control rabbit (Rabbit 68A, weighing 1,150 gms.) inoculated by the same route fell ill on the 20th day following upon infection, and died on the 25th day. Characteristic lesions were found throughout the central nervous system on microscopic examination.

Rabbit 223A was therefore immune against infection with the strain of *equine* origin. On the 12.5.27 (i.e. 202 days after the first inoculation, and 148 days after the second inoculation with the equine strain, it was inoculated intracerebrally with the strain of *ovine* origin. At the same time a control rabbit was similarly inoculated. Rabbit 223A continued in health, and gained in weight. The control showed symptoms of Borna disease 20 days after the inoculation, and died 27 days after the injection. The usual characteristic lesions were found in sections of the central nervous system of this control rabbit, and a portion of the brain infected another rabbit.

Experiment 2. The fact that repeated inoculations of fresh virus into the veins of rabbits may immunize them against subsequent intracerebral inoculation has already been referred to (see p. 71). We prepared rabbits by vaccinating in this way with a virus of *ovine* origin.[1] A fresh virulent emulsion of brain was centrifugalized for 5 minutes. The supernatant fluid was carefully pipetted off and inoculated in the marginal vein of the ear of rabbits, care being taken that the injection was carried out very slowly.

Rabbit 67. Weight 1,580 gms.

12.5.27. *First injection* of 2 c.cms. of a virulent centrifugalized emulsion into the vein.
16.5.27. Weight 1,600 gms. *Second injection* of 3 c.cms. of a virulent centrifugalized emulsion into the vein.
21.5.27. Weight 1,680 gms. Animal normal.
30.5.27. Weight 1,700 gms. Weight normal.
5.6.27. Weight 1,800 gms. Animal normal.
7.6.27. *Third injection* of 5 c.cms. of a virulent centrifugalized emulsion into the vein.

[1] Several animals were prepared in the same way, but we give below the protocol of the rabbit which served for an experiment of cross immunity between the equine and ovine strain.

8.6.27. Weight 1,690 gms. Animal normal.
13.6.27. Weight 1,820 gms. *Fourth injection* of 3 c.cms. of a virulent centrifugalized emulsion into the vein.
22.6.27. Weight 1,870 gms. Animal normal.

On 23.6.67 the animal, together with a control rabbit, was inoculated intracerebrally with an emulsion of fresh virus of *equine* origin.

Rabbit 67. Weight 1,870 gms.

30.6.27.	Animal normal.	Weight	1,980 gms.
7.7.27.	,,	,,	2,050 ,,
14.7.27.	,,	,,	2,200 ,,
20.7.27.	,,	,,	2,150 ,,
25.7.27.	,,	,,	2,100 ,,
28.7.27.	,,	,,	2,000 ,,
15.8.27.	,,	,,	2,100 ,,
29.8.27.	,,	,,	2,150 ,,

Control.

Rabbit 178B. Weight 1,770 gms.

30.6.27. Normal. Weight 1,820 gms.
7.7.27. ,, ,, 1,850 ,,
14.7.27. ,, ,, 1,800 ,,
18.7.27. Commencement of the disease. Weight 1,650 gms.
20.7.27. Typical symptoms of the disease. Weight 1,540 gms.
25.7.27. Severely ill. Weight 1,300 gms.
26.7.27. Found dead 33rd day after inoculation.

Microscopical examination of sections of the central nervous system—characteristic lesions.

This experiment shows that the virus of *ovine* origin immunizes against that of *equine* origin.

Experiment 3. A rabbit (81s) which had resisted inoculation with virulent virus of *ovine* origin, by scarification of the cornea, was inoculated intracerebrally 110 days later with fresh rabbit passage virus of *equine* origin. At the same time a control rabbit (25A) was inoculated intracerebrally with the same fresh passage virus. Rabbit 81s remained well, while the control, 25A, showed typical symptoms of Borna disease 32 days after inoculation, and died on the 48th day. In this case also the virus of ovine origin immunized Rabbit 81s against the pathogenic action of the virus of equine origin introduced into the brain.

Conclusion. From the above experiments the following conclusions can be drawn:

(1) Rabbits which have become resistant to the virus of enzootic encephalo-myelitis of *equine* origin prove also to be refractory to infection by intracerebral inoculation of the virus of *ovine* origin; the converse is also true.

(2) Rabbits which have become immunized against enzootic encephalo-myelitis keep this acquired immunity for at least 148 days. If the immunity is reinforced by repeated inoculations it may last at least 263 days.

V. Experiments on Cross Immunity between the Virus of Enzootic Encephalo-myelitis and other Viruses of the Filter-passing Group.

A. *Herpes.*[1]

On the basis of the following experiment we arrived at the conclusion published as a preliminary note in the *British Journal of Experimental Pathology* (1927) that rabbits immunized against the virus of herpes are still susceptible to infection with the virus of Borna disease.

Four large rabbits, 111A, 183A, 185A, and 187A were immunized against herpes. On 7.6.27 their immunity was tested by the intracerebral inoculation of fresh herpetic virus; they resisted such infection, while the control rabbit, No. 148A, infected by the same route, died on the 6th day. Sixteen days later the four surviving rabbits were inoculated intracerebrally with the virus of encephalomyelitis, together with a control rabbit, No. 182A. All the rabbits showed symptoms typical of experimental Borna disease and died; the results are recorded below.

No. of rabbit.	*Date of death.*	*Culture of brain.*	*Lesions.*
111A	Died on the 40th day after inoculation	Negative	Typical.
183A	,, ,, 32nd ,, ,, ,,	,,	,,
185A	,, ,, 31st ,, ,, ,,	,,	,,
187A	,, ,, 32nd ,, ,, ,,	,,	,,
Control Rabbit 182A	,, ,, 40th ,, ,, ,,	,,	,,

Conclusion. No cross immunity exists between herpes and enzootic encephalo-myelitis.

B. *Rabies.*[2]

On 9.9.27 two rabbits proved to have been immunized against the virus of Borna disease (Rabbit 223A, see p. 75, and Rabbit 67, see p. 75), together with, as a control, Rabbit 41A, were inoculated intracerebrally with a 'street' virus of rabies. The two rabbits, 223A and 67, which were immune to Borna disease, became paralysed on the 8th day after the inoculation with rabies and died on the 10th day; cultures from the brain were negative. Negri bodies were found in sections of the Ammon's horn of both rabbits. The control rabbit became paralysed after 8 days and died on the 12th day; Negri bodies were found in sections of the Ammon's horn.

Conclusion. No cross immunity exists between rabies and enzootic encephalo-myelitis.

[1] We thank Dr. Perdrau, of the National Institute for Medical Research, for kindly putting a strain of this virus at our disposal.

[2] We thank Dr. Manouelian of the Pasteur Institute, Paris, for his kindness in putting this strain at our disposal.

C. *Polio-myelitis.*[1]

In the experiments with this virus two strains have been used, (1) a strain of low virulence kindly furnished by Professor Mac-Intosh, (2) a very virulent strain kindly provided by Professor Petit. This latter strain, which has been utilized by us in another series of experiments, killed monkeys by intracerebral inoculation as indicated below:

TABLE XIII.

Monkey (*Macacus rhesus*)			No. 5.	Died the 9th day after inoculation.				
,,	,,	,,	No. 6.	,,	12th	,,	,,	,,
,,	,,	,,	No. 13.	,,	11th	,,	,,	,,
,,	,,	,,	No. 15.	,,	11th	,,	,,	,,

The lesions produced by this strain of polio-myelitis in the central nervous system of monkeys were very intense, neuronophagia was frequently found in the anterior horns of the spinal cord as well as in the paravertebral ganglia.

The virus of polio-myelitis taken from monkeys infected experimentally is generally considered as non-pathogenic for the rabbit.[2]

In a first series of experiments we gave repeated intracerebral inoculations of polio-myelitis to young rabbits; some time afterwards the rabbits so prepared were infected by the same route with the virus of enzootic encephalo-myelitis.

Table XIV gives the results of this experiment.

Thus the four rabbits having received three successive intracerebral inoculations with the virus of polio-myelitis and one rabbit which received no such inoculation, proved to be still susceptible to infection with the virus of Borna disease.

The conclusion that absolutely no cross immunity exists between the virus of polio-myelitis and that of Borna disease, however, is weakened by the following experiment on a monkey.

Monkey M. 2 (*Macacus rhesus*), which had apparently almost completely recovered from an intracerebral inoculation of the virus of Borna disease, after having shown the morbid symptoms recorded on p. 36, was inoculated into the brain with a passage virus of polio-myelitis (strain Petit). At the same time two controls of a comparable size and the same species were similarly inoculated. The subsequent history of the two controls was as follows:

M. 13 (*Macacus rhesus*)

5.10.27. Inoculated intracerebrally with the virus of polio-myelitis.
10.10.27. Normal.
11.10.27. Paralysed, found procumbent.
12.10.27. ,, ,, ,,

[1] Our thanks are due to M. le Prof. A. Petit, of the Pasteur Institute, and Prof. Mac-Intosh of the Bland Sutton Institute for supplying us with strains of polio-myelitis virus.

[2] Krause and Meinicke (1909) and also Dahm (1909), however, have recorded a few experiments giving positive results, using virus taken directly from human cases, but the general opinion held to-day is that the virus of polio-myelitis is not pathogenic for rabbits.

TABLE XIV.

No. of rabbit.	*23.5.27.* *1st inoculation, virus MacIntosh.*	*4.8.27.* *2nd inoculation, virus MacIntosh.*	*6.9.27.* *3rd inoculation, virus Petit.*	*20.9.27.* *Brain with Borna.*	*Results.*
	Weight in gms.	*Weight in gms.*	*Weight in gms.*	*Weight in gms.*	
103A	660	1,070	1,250	1,310	First symptoms 22nd day after inoculation. Died the 32nd day. Lesions in C.N.S. typical.
104A	620	1,250	1,380	1,530	First symptoms 22nd day after inoculation. Died the 36th day. Lesions in C.N.S. typical.
105A	600	1,200	1,250	1,390	First symptoms 16th day after inoculation. Died 23rd day. Lesions in C.N.S. typical.
110A	720	1,200	1,380	1,460	First symptoms 25th day. Died 32nd day. Lesions in C.N.S. typical.
26A	—	—	1,870	1,900	First symptoms 16th day. Died 29th day. Lesions in C.N.S. typical.
68A	Control	—	—	2,620	First symptoms 20th day. Died 28th day. Lesions in C.N.S. typical.
69A	,,	—	—	2,650	First symptoms 20th day. Died 28th day. Lesions in C.N.S. typical.

13.10.27. Paralysed, found procumbent.
14.10.27. ,, ,, ,,
15.10.27. ,, ,, ,,
16.10.27. Found dead, 11th days after inoculation.
Cultures of brain. Negative.
Sections. Lesions typical of polio-myelitis.

M. 15 (*Macacus rhesus*)

5.10.27. Inoculated intracerebrally with the virus of polio-myelitis.
10.10.27. Normal.
11.10.27. ,,
12.10.27. ,,
13.10.27. ,,
14.10.27. Paralysis of the left arm.
15.10.27. Found procumbent and paralysed.
16.10.27. Found dead 11th day.
Cultures of brain. Negative.
Sections. Lesions typical of polio-myelitis.

Monkey M. 2 (*Macacus rhesus*), which had survived the inoculation with Borna disease, when inoculated with polio-myelitis, behaved as follows:

5.10.27. Inoculated into the brain with the virus of polio-myelitis 130 days after the inoculation with the virus of Borna disease.
10.10.27. No added symptoms up to this date.
15.10.27. ,, ,, ,, ,,
17.10.27. ,, ,, ,, ,,
19.10.27. ,, ,, ,, ,,
20.10.27. Fifteenth day, myoclonic movements.
21.10.27. Epileptiform trembling movements.
22.10.27. Slight paresis of the hind quarters.
23.10.27. Paresis more marked.
24.10.27. Total paralysis of the hind quarters and partial paralysis of the fore-limbs.
25.10.27. Could make movements with the head, but the total paralysis of the arms and legs prevented it from getting up.
26.10.27. Same condition.
27.10.27. Same condition, but still feeding.
28.10.27. ,, ,, ,,
29.10.27. Animal in a comatose state.
30.10.27. ,, ,,
31.10.27. Died 26 days after the inoculation with the virus of polio-myelitis and 156 days after the infection with Borna.

We have drawn attention in the course of this monograph to the resemblance between the symptomatology and histological picture in polio-myelitis in the monkey and experimental enzootic encephalo-myelitis in the same species. The differences between them are the longer period of incubation and the slower evolution and less intensity of the lesions in the nervous system in the latter disease.

Monkey M. 2 died after a lapse of time much longer than the average for animals inoculated with polio-myelitis, the strain used generally killing the species of animal in from 9 to 11 days. We had, therefore, to determine whether it died from polio-myelitis or from a persisting infection of Borna disease. On an anatomo-pathological

study of the various parts of the nervous system of this monkey the lesions of experimental enzootic encephalo-myelitis were found, including the corpuscles of Joest-Degen. The general aspect of the alterations in the lumbar region of the spinal cord indicated that these were produced by the virus of Borna disease and not by the virus of polio-myelitis. This conclusion received further support when emulsions from various parts of the nervous system were passaged to fresh animals. The results of these inoculations were as follows:

(1) Rabbit 460A, weighing 2,320 gms., and Rabbit 462A, weighing 1,740 gms., were inoculated intracerebrally with an emulsion of the brain of Monkey M. 2. The former died on the 37th day and the latter on the 38th day after infection, and lesions characteristic of experimental Borna disease were found in sections of the various parts of the nervous system of both animals. A passage was made with the brain of Rabbit 462A to a fresh Rabbit 405A, weighing 1,330 gms. Rabbit 405A succumbed on the 42nd day of Borna disease.

(2) Rabbit 464A, weighing 1,960 gms., Rabbit 458A, weighing 1,760 gms., and Rabbit 459A weighing 2,500 gms., were inoculated intracerebrally with an emulsion of the cervical region of the spinal cord of Monkey M. 2. Rabbit 464A died on the 34th day, 458A on the 30th day, and 459A on the 30th day after inoculation, and lesions typical of Borna disease were found in sections of the nervous system of all three. A passage was made with the brain of Rabbit 458A to a fresh rabbit, 414A, which died on the 33rd day of experimental enzootic encephalo-myelitis.

(3) Rabbit 454A, weighing 1,960 gms., Rabbit 455A, weighing 1,960 gms., and Rabbit 463A, weighing 2,300 gms., were inoculated intracerebrally with an emulsion of the lumbar cord of Monkey M. 2. The first died on the 40th day, the second on the 35th day, and the third on the 34th day of experimental Borna disease. Rabbit 415A, weighing 1,880 gms., when inoculated intracerebrally with an emulsion of the brain of Rabbit 463A, died of Borna disease on the 30th day.

The results of these rabbit inoculations proved that the virus of Borna disease still existed in the brain and spinal cord of Monkey M. 2.

Monkey M. 20 (*Macacus rhesus*) was inoculated with an emulsion of a portion of the brain and the cervical cord of Monkey M. 2. It remained healthy for a period of 60 days, when the commencement of paresis of the hind quarters was first observed. Paresis later became accentuated and reached the fore limbs. The animal died on the 68th day after inoculation, and the lesions of experimental enzootic encephalo-myelitis were found in sections of the central nervous system. Rabbits 340A and 341A were inoculated intracerebrally with an emulsion of the brain of Monkey M. 20, and both died of experimental Borna disease.

In *résumé*, the activity of virus of Borna disease which had become dormant in the central nervous system of Monkey M. 21, 130 days after the inoculation, as indicated by the clinical picture, was revived by the introduction of the virus of polio-myelitis by the intracerebral route. As a result of the second infection the monkey died and we

demonstrated the presence of the virus of Borna disease in its brain and spinal cord, but the virus of polio-myelitis had been destroyed.

Without wishing to attach too much importance to this single experiment, we are forced to conclude that the monkey which survived for 130 days the infection with Borna disease showed an exceptional resistance of an anomalous character to the virus of polio-myelitis. This resistance might be the result of a certain degree of immunity conferred by the first infection. On the other hand, it might be explained in another way. The experiments by Gildemeister and Herzberg (1925) indicate that guinea-pigs which showed lesions on the pads of the metatarsus as a result of infection with the virus of vaccinia develop a certain local resistance to infection with herpetic virus inoculated by the same route. The authors conclude that there is a certain degree of immunity conferred by one virus against infection with the other. In a similar way, certain rabbits, which after recovering from vaccinal keratitis, when inoculated some time later on the same cornea with the virus of herpetic encephalitis, sometimes resist the second infection.

We do not consider in these circumstances that an immunity results in the proper sense of the word, but rather a local mobilization of the elements of defence provoked by the first virus inoculated, thus conferring on the tissue a certain degree of resistance of a non-specific character which does not exist normally.

We intend to repeat our experiment. In addition, monkeys are being immunized against polio-myelitis which will be inoculated later with the virus of Borna disease.

VI. Summary.

A solid immunity can occasionally be obtained against Borna disease in the rabbit by inoculating suitably attenuated virus into the brain.

Multiple intravenous injections, infection by corneal scarification, or intratesticular inoculation with fresh virus, can also produce immunity.

We have not succeeded in producing immunity by inoculating virus killed by chloroform, ether, or ultra-violet light intracerebrally into rabbits.

Multiple inoculations subcutaneously of large quantities of formolized virus leads to immunity in a limited number of animals.

Rabbits immunized against an equine strain of the virus of Borna disease were resistant to intracerebral infection with an ovine strain and vice versa.

No cross immunity was obtained between Borna disease and herpes or rabies. Cross immunity between Borna disease and polio-myelitis was not observed when rabbits were the subject of experiment, but in an experiment carried out on a monkey the result suggested that some resistance to the virus of polio-myelitis may be produced by a previous attack of experimental Borna disease.

13. CHEMOTHERAPY

Various medicaments, calomel, mercuric chloride, salvarsan, atoxyl, have been tried in the treatment of enzootic encephalo-myelitis. The greatest success as regards the treatment by drugs has been claimed for the administration of urotropine (hexamethylenetetramine). Moussu and Marchand (1926) obtained remarkably good results in the treatment of the disease in horses and cattle by inoculation of urotropine intravenously. According to these authors, when 15 to 20 gms. is administered on the appearance of the first symptoms, the mortality in epizootics may be lowered from 80 or 90 per cent. to 25 per cent. In Germany Ostertag (1924) has generalized the use of urotropine on a large scale in the treatment of enzootic encephalo-myelitis. H. Bohn (1927) inoculated 30 gms. per day for 4 to 5 days intravenously into affected horses with good results. Trepel (1926) advocates the use of a total of 100 gms. during the course of a few days. Grimm (1927) obtained a smaller number of recoveries than the authors cited above. All authors appear to be agreed as to the beneficial action of the drug in Borna disease of the horse.

We have carried out experiments to see whether urotropine was as efficacious in treating the disease in the rabbit as in the horse. We commenced by determining the maximum intravenous dose supported by a rabbit. This appeared to be about 0·5 gms. per kgm., a dose which can be repeated at least eight times at 4 to 5 days' interval. Subsequently the experiment recorded in Table XV was carried out.

Summary. The dose of urotropine we gave to the rabbit was equivalent, weight for weight, to 100–200 gms. for the horse and to a total amount of 600 gms., yet we were not able to demonstrate any prophylactic or curative action.

TABLE XV.

No. of rabbit.	*Inoculation of virus intracerebrally.* 23.6.27. *Urotropine inoculated into veins.*	28.6.27. *Urotropine inoculated into veins.*	2.7.27. *Urotropine inoculated into veins.*	6.7.27. *Urotropine inoculated into veins.*	11.7.27. *Urotropine inoculated into veins.*	19.7.27. *Urotropine inoculated into veins.*	24.7.27.	Result.
180A	Weight 1,890 gms. Urotropine 0·8 gr.	Weight 2,020 gms. Urotropine 0·8 gr.	Weight 1,950 gms. Urotropine 0·8 gr.	Weight 1,900 gms. Urotropine 0·8 gr.	Weight 1,900 gms. Urotropine 0·8 gr.	Animal showing symptoms. Weight 1,880 gms.	Typical symptoms. Weight 1,550 gms.	Died of disease 39th day.
181A	Weight 2,100 gms. Urotropine 0·8 gr.	Weight 2,150 gms. Urotropine 0·8 gr.	Weight 2,100 gms. Urotropine 0·8 gr.	Weight 2,050 gms. Urotropine 0·8 gr.	Weight 2,000 gms. Urotropine 0·8 gr.	Weight 1,900 gms. Typical disease. Urotropine 0·8 gr.	Typical disease. Weight 1,600 gms.	Died of disease 32nd day.
179A	Weight 2,220 gms. Urotropine 0·8 gr.	Weight 2,200 gms. Urotropine 0·8 gr.	Weight 2,250 gms. Urotropine 0·8 gr.	Weight 2,300 gms. Urotropine 0·8 gr.	Weight 2,200 gms. Urotropine 0·8 gr.	Typical commencement of disease. Weight 2,100 gms. Urotropine 0·8 gr.	Typical disease. Weight 1,650 gms.	Died of disease 32nd day.
Control 178A	Virus into brain. Weight 1,770 gms. No urotropine	Weight 1,820 gms. No urotropine	Weight 1,850 gms. No urotropine	Weight 1,850 gms. No urotropine	Weight 1,800 gms. No urotropine	Typical symptoms Weight 1,600 gms. No urotropine	Typical disease. Weight 1,300 gms.	Died of disease 34th day.
Control 187A	Not inoculated with virus. Weight 2,400 gms. Urotropine 1 gr.	Weight 2,350 gms. Urotropine 1 gr.	Weight 2,380 gms. Urotropine 1 gr.	Weight 2,320 gms. Urotropine 1 gr.	Weight 2,400 gms. —	Weight 2,280 gms. Urotropine 1 gr.	—	Survived.

REFERENCES

Albertin, A. (1926).—Thesis. Lyons.
Alvisatos, G. P. (1922). *Deutsche med. Wchnschr.*, **48**, 295.
Baudoin, A., and Schaeffer, H. (1926). *Paris Méd.*, **16**, 249.
Beck, A. (1925). *Ztschr. f. Infektionsk. d. Haustiere*, **28**, 99.
Beck, A., and Frohböse (1926). *Archiv f. wissensch. u. prakt. Tierh.*, **54**, 84.
Bériel, L. (1926).—*Lyon méd.*, **1**, 667.
Bériel, L., and Devic, A. (1926). *Lyon méd.*, **1**, 671.
Bohn, H. (1927). *Deutsche tierärztl. Wchnschr.*, **35**, 584.
Causel, M. (1924). *Rec. méd. vét.*, **100**, 526.
Christiani (1909). *Arch. f. wissensch. u. prakt. Tierh.*, **35**, 253.
Dahm (1909). *München. med. Wchnschr.*, **56**, 2553.
David and Dekester (1926). *Bull. et mém. Soc. méd. d'hôp. de Paris*, 3, S., **50**, 75.
Dexler, H. (1900). *Ztschr. f. Thiermed.*, **4**, 110.
Dioby, K. (1919). *Immunity in Health.* Oxford Medical Publications.
Dobberstein, J. (1925). *Berl. tierärztl. Wchnschr.*, **41**, 177.
Eichbaum, Stöhr, and Wilke (1865–6). *Mitt. d. tierärzt. Prax. in preuss. Staat.* Berlin, 135.
Ellinger, R. (1927). *Tierärztl. Rdsch.*, 33, 201.
Ernst, W. (1925). *München. tierärztl. Wchnschr.*, **76**, 477.
Ernst, W., and Hahn, H. (1926). *München. tierärztl. Wchnschr.*, **77**, 46.
—— (1927). *München. tierärztl. Wchnschr.*, **78**, 85.
Flexner, S., and Amos, H. L. (1917). *J. Exper. Med.*, **25**, 525.
Foerster cited by Marinesco and Draganesco.
Gildemeister, E., and Herzberg, K. (1925). *Deutsche med. Wchnschr.*, **51**, 97.
Glamser (1926). *Deutsche tierärztl. Wchnschr.*, **34**, 312.
Grimm (1927). *Berl. tierärztl. Wchnschr.* (1927), **43**, 153.
Head and Campbell (1900). *Brain*, **23**, 353.
Hempt, A. (1925). *Ann. de l'Inst. Pasteur*, **39**, 632.
Heydt (1914). *Deutsche tierärztl. Wchnschr.*, 249.
Hutyra and Marek (1922). *Spezielle Pathologie u. Therapie d. Haustiere*, 6th Ed. Jena.
Joest, E. (1921). *Ztschr. f. Infektionskr. d. Haustiere*, **21**, 97.
—— (1926). *Klin. Wchnschr.*, **5**, 209.
Joest, E., and Degen, K. (1909). *Ztschr. f. Infektionskr. d. Haustiere*, **6**, 348.
—— (1911). *Ztschr. f. Infektionskr. d. Haustiere*, **9**, 1.
—— (1911). *Deutsche Ztschr. f. Nervenh.*, **42**, 293.
Johne (1897, 1). *Deutsche Ztschr. f. Thiermed.*, **22**, 369.
—— (1897, 2). *Ber. u. d. Veterinärw. im Königr. Sachs.*
Kahlmeter, G. (1927). *Acta med. Scandinav.*, **65**, 709.
Kragerud, A., and Gunderson, T. (1921). *Norges offisielle statistik.*, **7**, 15.
Kraus, R., Kantor, L., Fischer, H., and Quiroga, R. (1920). *Ztschr. f. Immunitätsforsch.*, **30**, 121.
Krause, P., and Meinicke, E. (1909). *Deutsche med. Wchnschr.*, **35**, No. 12.
Lessage, J., and Frisson, M. (1912). *Rev. gén. de méd. vét.*, **20**, 657.
Levaditi, C. (1926). *L'herpes et le zona.* Masson et Cie, Paris.
Levaditi, C., and Nicolau, S. (1923). *Ann. d. l'Inst. Pasteur*, **37**, 1.
—— (1922). *Compt. rend. Acad. d. sc.*, **174**, 778.
Levaditi, C., Harvier, P., and Nicolau, S. (1921). *Compt. rend. Soc. de biol.*, **84**, 524.
—— (1922). *Ann. d. l'Inst. Pasteur*, **36**, 63 and 105.
Lohr (1910). *Beitr. z. Bakt. d. Gehirnruckenmarksseuche d. Pferde.* Inaug. Diss. (Leipzig). Dresden.
Manouelian, Y., and Viala, J. (1926). *Compt. rend. Acad. d. sc.*, **183**, 1357.
—— (1927). *Compt. rend. Acad. d. sc.*, **184**, 630.
Marchand, L., and Moussu, R. (1924). *Compt. rend. Acad. d. sc.*, **178**, 149.
Marcq, J. (1909). *Ann. de méd. vét.*, **58**, 11.
Marie, A., and Mutermilch, S. (1927). *Compt. rend. Acad. d. sc.*, **184**, 911.
Marinesco, G., and Draganesco, S. (1927). *Bull. Acad. de méd.*, **97**, 473.
Miessner, H. (1926). *Deutsche tierärztl. Wchnschr.*, **34**, 637.
Moussu, R. (1922). *Rec. de méd. vét.*, **98**, 261.
—— (1926). *Recherches sur certain affections du syst. nerveux central des animaux domestiques.* Thesis, Vigot Frères, Paris.
Moussu, R., and Marchand, L. (1924). *Rec. de méd. vét.*, **100**, 5; 65.
Nicolau, S., and Galloway, I. A. (1927). *Brit. J. Exper. Path.*, **8**, 336.
Noack (1908). *Ber. u. d. Veterinärw. im Königr. Sachs.*, p. 41.

Oppenheim, H. (1907). *Ztschr. f. Infektionskr. d. Haustiere*, **2**, 148.
Ostertag (1900). *Berl. tierärztl. Wchnschr.*, 433.
—— (1906). *Berl. tierärztl. Wchnschr.*, 622.
—— (1924). *Berl. tierärztl. Wchnschr.*, **40**, 705.
Péhu, M., and Dechaume, J. (1927). *Ann. de méd.*, **22**, 172.
Petiot (1921–2). Thesis (Paris).
Pette cited by Marienesco and Draganesco.
Popow (1882), cited by Hutyra and Marek.
Priemer (1925) cited in the thesis of Schmidt. Leipzig (1925).
Prietsch (1896). *Ber. u. d Veterinärw. im Königr. Sachs.*, p. 126.
Pröger (1896). *Ber. u. d. Veterinärw. im Königr. Sachs.*, p. 105.
Rautmann, H. (1926). *Tierärztl. Rdsch.*, **32**, 841.
Ravina, A. (1927). *Presse méd.*, **35**, 646.
Remlinger, P. (1919). *Compt. rend. Soc. de biol.*, **82**, 52.
Roch, M., and Bickel, G. (1927). *Schweiz. med. Wchnschr.*, **57**, 18.
Roloff (1868). *Mitt. a. d. tierärztl. Prax. in preuss. Staat.* Berlin, 135.
Savigné, A., and Leblanc, P. (1897). *J. méd. vét. et de zootech.*, 1897, 5 S., **1**, 274.
Schmidt (1870). *Mag. f. d. ges. Tierh.*, Berlin, 186.
Siedamgrotzky and Schlegel (1896). *Arch. f. wissensch. u. prakt. Thierh.*, **22**, 287.
Skoog, A. L. (1923). *Med. Clin. North Amer.*, **7**, 1323.
Spiegl, A. (1922). *Ztschr. f. Infektionskr. d. Haustiere*, **23**, 147.
Targowla, J. (1894). *Rev. Neurol.*, **2**, 465.
Thomas, A., and Rendu (1925). *Rev. Neurol.*, **2**, 758.
Thomas, H. M. (1898). *Phila. M. J.*, **1**, 885.
Trepel (1926). *Berl. tierärztl. Wchnschr.*, **42**, 881.
Van Gehuchten, A., and Nelis, G. (1900). *Presse. méd.*, **8**, 113.
Viets, H. R. (1927). *Arch. Neurol. and Psychiat.*, **17**, 794.
Waldmann, J., and Pape, J. (1921). *Berl. tierärztl. Wchnschr.*, **37**, 449.
Walther (1899). *Ber. u. d. Veterinärw. im Königr. Sachs.*, p. 80.
Wischnikowitch (1889) cited by Hutyra and Marek.
Wohlwill, F. (1924). *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **89**, 171.
Wörz cited by Hutyra and Marek (see above).
Zwick, W. (1925). *Berl. tierärztl. Wchnschr.*, **41**, 453.
—— (1926). *Seuchenbekampfung*, **3**, 57.
Zwick, W., and Seifried, O. (1924). *Berl. tierärztl. Wchnschr.*, **40**, 465.
—— —— (1925). *Berl. tierärztl. Wchnschr.*, **41**, 129.
Zwick, W., Seifried, O., and Witte, J. (1926). *Ztschr. f. Infektionskr. d. Haustiere*, **30**, 42.

DESCRIPTION OF PHOTOGRAPHS AND COLOURED PLATES

Fig. 1. Photograph of Rabbit No. 275. 29 days after intracerebral infection with the virus of Borna disease, and 3 days before death. The characteristic position of the head and ears is depicted.

Fig. 2. Photograph of *Macacus rhesus* No. 1. 64 days after inoculation. Paralysis of the hind quarters; paresis of the right arm, the hand not being able to grip any object presented to it.

Fig. 3. Photograph of *Macacus rhesus* No. 1. 69 days after inoculation. Characteristic 'hunched up' attitude.

Fig. 4. Photograph of *Macacus rhesus* No. 2. 51 days after the infection by the intracerebral route. Facial paralysis on the left side.

Fig. 5. Photograph of *Macacus rhesus* No. 2. 67 days after inoculation. 3rd crisis of the disease. Facial paralysis on the right side.

Fig. 6. Photograph of Guinea-pig 95K. 60 days after intracerebral inoculation, and 5 days before death. Paralysis of the hind quarters.

Fig. 7. Photograph of Guinea-pig 1A. 50 days after intracerebral inoculation, and 2 days before death. General paralysis.

Fig. 8. Photograph of Rat No. 14. 63 days after inoculation intracerebrally, and 4 days before the death of the animal. At this stage the rat placed on the flank made several useless efforts with the fore-legs to recover its normal position.

Fig. 9. Photograph of the stomach wall of Rabbit 212 which died 44 days after the intracerebral inoculation, showing the haemorrhagic lenticular areas. L = greater curvature of the stomach; P = lenticular haemorrhagic areas.

Fig. 10. Microphotograph, ×160. Rabbit 209 dead 71 days after intratesticular inoculation. Meningitis with mononuclear cells with 'cuffing' around the vessels. V = lumen of blood-vessel; C = cerebral cortex; M = mononuclear leucocyte of inflammatory process.

Fig. 11. Microphotograph, ×300. Rabbit 51A dead 28 days after intracerebral infection. Perivascular 'cuff' in the cerebral cortex cut longitudinally. V = lumen of blood-vessel; E = endothelium of blood-vessel; M = mononuclear leucocytes taking part in the infiltrative process.

Fig. 12. Microphotograph, ×165. Rabbit 11D dead 27 days after intracerebral inoculation. Section showing the aspect of the *Cornu Ammonis*. F = area of infiltrating lymphocytes between the nerve-cells; L = infiltrating lymphocytes; C = cells containing the corpuscles of Joest-Degen within their nuclei.

Fig. 13. Microphotograph, ×800. Rabbit 25 dead 35 days after cerebral infection. '*Cyst*' in a nerve-cell of the Ammon's horn. C = 'cyst'; N = nucleus; n = nucleolus.

Fig. 14. Microphotograph, ×1,000. Rabbit 80A dead 28 days after inoculation intracerebrally. 'Cyst' in the protoplasm of a nerve-cell of the Ammon's horn. C = 'cyst'; N = nucleolus.

Fig. 15. Microphotograph, ×900. Rabbit 11D dead 27 days after cerebral infection. 'Cyst' in the protoplasm of a nerve-cell of the *Cornu Ammonis*.

Fig. 16. Microphotograph, ×1,000. Rabbit 51A dead 28 days after inoculation into the brain. 'Cyst' in the protoplasm of a nerve-cell in the *Cornu Ammonis*.

Fig. 17. Microphotograph, ×1,000. Rabbit No. 22 dead 24 days after cerebral infection. Nerve-cell in medulla oblongata showing neuronophagia. C = degenerated cytoplasm; p = polymorphonuclear penetrating neuron; m = mononuclear cells.

Fig. 18. Microphotograph, ×230 (enlarged two diameters). Rabbit 295 dead 19 days after intracerebral inoculation. Section in the region of the pons showing (d) degenerated nerve-cells, (s) '*satellitism*' with commencing neuronophagia, (n) neuronophagia, (l) lymphocytes in the parenchyma.

FIG. 19. Microphotograph, ×1,000. Rabbit 278 inoculated intracerebrally and sacrificed 20 days later. Commencing neuronophagia in the anterior horn of the spinal cord in the lumbar region. N = nucleus of nerve-cell: P = cytoplasm; M = mononuclear cells.

FIG. 20. Microphotograph, ×66. Rabbit 44 dead 33 days after intracerebral inoculation. Section of the lumbar region of the spinal cord. S = cord; M = perivascular 'cuff' in a nerve-trunk; I = interstitial mononuclear infiltration; A = accumulation of mononuclear leucocytes.

FIG. 21. Microphotograph, × 66. Rabbit No. 30 dead 36 days after the inoculation given intracerebrally. Spinal ganglion in the lumbar region. N = ganglion nerve-cell in a state of neuronophagia; I = formation of a nodule of mononuclear leucocytes which has formed at the site where a nerve-cell is undergoing neuronophagia; V = perivascular infiltration.

FIG. 22. Microphotograph, ×66. Rabbit 50A dead 53 days after cerebral inoculation. Section of the cord in the lumbar region including a spinal ganglion. V = vacuolization in certain ganglion cells; I = interstitial infiltration; N = neuronophagia; P = perivascular infiltration.

FIG. 23. Microphotograph, ×1,000. Rabbit 164 dead 29 days after intracerebral inoculation. Spinal ganglion in the lumbar region. Neuronophagia with the formation of nodules consisting of mononuclear cells. R = remains of a destroyed neuron; V = vacuole in a nerve-cell; P = polymorphonuclear; L = lymphocytes.

FIG. 24. Microphotograph, ×950. Rabbit No. 30 dead 36 days after intracerebral inoculation. Spinal ganglion in the lumbar region. Degeneration of a ganglion cell and commencing neuronophagia. C = degenerated cell which has become oxyphilic and is on the way to destruction; P = polymorphonuclear cells; L = lymphocytes; M = mononuclears participating in the process of neuronophagia.

FIG. 25. Microphotograph, ×1,000. Rabbit 164 dead 29 days after intracerebral inoculation. Spinal ganglion in the thoracic region. N = neuronophagia; G = particle of chromatin probably originating from a pyknosed polymorphonuclear; M = macrophage; C = cell—commencing neuronophagia; L = lymphocytes.

FIG. 26. Microphotograph, ×800. Rabbit No. 30 dead 36 days after cerebral inoculation. Spinal ganglion in the lumbar region. M = mononuclear cells infiltrating; D = commencing neuronophagia; V = vessel surrounded by perivascular 'cuff'.

FIG. 27. Microphotograph, ×5. Rabbit 140A dead 49 days after intracerebral infection with glycerinated virus of passage. Section of the terminal part of the spinal cord with roots of the sciatic nerve and corresponding ganglion. M = spinal cord; R = nerve-root; G = spinal ganglion; S = sciatic nerve. In sections cut in this way the lesions in the regions mentioned above can be seen as a whole.

FIG. 28. Microphotograph, ×350. Rabbit 140A. Longitudinal section of the sciatic nerve near the popliteal region. A perivascular 'cuff' is seen. V = lumen of vessel. M = infiltrating mononuclear cells; L = lymphocytes in the thickness of the nerve.

FIG. 29. Microphotograph, ×150. *Macacus rhesus* No. 1. Section of the parietal lobe; C = cerebral cortex; M = mononuclear meningitis; V = blood-vessel cut longitudinally with walls infiltrated with mononuclear cells.

FIG. 30. Microphotograph, ×120. *Macacus rhesus* No. 3. Perivascular cuffing in the frontal lobe of the cerebral cortex. V = lumen of vessel; P = perivascular infiltration.

FIG. 31. Microphotograph, ×150. *Macacus rhesus* No. 1. Section of the parietal lobe of the cerebral cortex. Pseudo gumma (nodule) produced by mononuclear cells, with a small vessel in the centre (very intense process of perivascular infiltration). V = blood-vessel; E = nodule formed by mononuclear cells.

FIG. 32. Microphotograph, ×360. *Macacus rhesus* No. 1. Section through basal ganglia. Small area of mononuclear cells. N = neuron; L = lymphocytes.

FIG. 33. Microphotograph, ×150. *Macacus rhesus* No. 1. Vascular infiltration in the terminal part of the spinal cord. V = lumen of blood-vessel; M = muscular coat; P = infiltrating mononuclear cells.

FIG. 34. Microphotograph, ×360. *Macacus rhesus* No. 1. Posterior root of sciatic nerve. Interstitial infiltration and perivascular 'cuffing'. V = lumen of blood-vessel; M = mononuclear cell in the process of perivascular 'cuffing'; m = macrophage.

FIG. 35. Microphotograph, × 700. *Macacus rhesus* No. 3. Spinal ganglion in cervical region. Corpuscles (type Joest-Degen) surrounded by a halo in the nerve-cells of the ganglion. C = intranuclear corpuscle of Joest-Degen; N = nucleolus.

Fig. 36. Microphotograph, (×36, enlarged 2½ diameters). *Macacus rhesus* No. 1. Section of spinal ganglion in lumbar region of spinal cord showing the profound changes which predominate the peripheral parts of the nervous system. (i) = intranuclear corpuscle (Joest-Degen) with surrounding halo; n = nodule of mononuclear cells replacing destroyed neuron; d = degenerated ganglion cells; np = neuronophagia; ic = pericellular infiltration; it = interstitial infiltration; c = capsule of ganglion.

Fig. 37. Microphotograph, ×55. *Macacus rhesus* No. 3. Section through the brachial nerve. Perivascular 'cuffing' and interstitial infiltration. G = nerve sheath; V = perivascular 'cuffs'; I = interstitial infiltration.

Fig. 38. Microphotograph, ×460. *Macacus rhesus* No. 1. Transverse section of the sciatic nerve several centimetres from its exit from the greater sciatic foramen—perivascular 'cuff'. V = lumen of blood-vessel; E = vascular endothelium; L = lymphocytes.

Fig. 39. Microphotograph, ×55, (photograph enlarged 2½ diameters). *Macacus rhesus* No. 1. Transverse section of sciatic nerve after its exit from the greater sciatic foramen. V = large vessel with perivascular infiltration; v = small vessels showing same phenomenon; l = lymphocytes (interstitial infiltration).

COLOURED PLATES

PLATE I

Fig. 1. Staining, toluidin blue-eosin. Obj. 5 mm., oc. 2, ×260.
Rabbit 295 dead 19 days after intracerebral infection. Cellular degeneration in the medulla oblongata. N = neuron in normal state; C = degenerated nerve-cell—tigrolysis-oxyphilia—nucleus eccentric; E = degenerated neuron; F = fragment of nerve-cell; L = lymphocytes.

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4.
Rabbit 25 dead 35 days after cerebral inoculation. Oxyphilic corpuscle surrounded by a halo in a nerve-cell in the Ammon's horn. C = corpuscle (type Joest-Degen) surrounded by a halo.

Fig. 3. Staining, Mann. 1/12 oil immersion, oc. 4.
Rabbit 80A dead 28 days after the inoculation into the brain. N = nucleus of neuron repulsed by the 'cyst'; n = nucleolus; C = '*Cyst*', this '*cyst*' is the degenerated nucleus of a mononuclear cell which has succeeded in penetrating the nerve-cell.

Fig. 4. Staining, toluidin blue-eosin. 1/12 oil immersion, oc. 4.
Guinea-pig 85E dead 82 days after cerebral infection. Section of medulla oblongata. N = nucleolus; C = corpuscle of Joest-Degen; H = halo around the corpuscle.

Fig. 5. Staining, Mann. 1/12 oil immersion, oc. 4.
Rabbit 209 dead 71 days after the inoculation of the virus into the testicle. Neuron of the anterior horn of the spinal cord in the lumbar region. C = Intranuclear corpuscle (Joest-Degen); N = nucleolus.

PLATE II

Fig. 1. Staining, toluidin blue-eosin. 1/12 oil immersion, oc. 1.
Rabbit 243 dead 22 days after inoculation into the brain. Section of the cord anterior horn showing degeneration of the nerve-cells. V = small blood-vessel; P = protoplasm of degenerated nerve-cells; D = protoplasm debris; O = vacuole in the cytoplasm of a degenerated neuron; C = cytoplasm; L = lymphocytes.

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4.
Macacus rhesus No. 1. Neurons of the anterior horn of the thoracic region of the cord containing corpuscles (Joest-Degen type). N = nucleus of the nerve-cell; n = nucleolus; C = corpuscle (Joest-Degen) surrounded by a halo. At the periphery of the corpuscle a blue staining area is seen.

PLATE III

Fig. 1. Staining, Mann. 1/12 oil immersion, oc. 2.
Macacus rhesus No. 1. Pyramidal cells of the frontal lobe containing oxyphilic corpuscles surrounded by a halo. N = nucleolus; C = corpuscle (Joest-Degen).

Fig. 2. Staining, Mann. 1/12 oil immersion, oc. 4.
Rabbit 298 dead 39 days after intracerebral inoculation. Cervical region of the cord: anterior horn. Formation of intranuclear corpuscles in the interior of the nucleus of the neuron. N = nucleolus; C = oxyphilic corpuscles; a study of the process of the

formation of the corpuscles of Joest-Degen has shown that several small oxyphilic corpuscles fuse together to form one large corpuscle surrounded by a halo.

FIG. 3. Staining, orange G. eosin, polychrome blue (Unna). 1/12 oil immersion, oc. 4.
Guinea-pig 99K dead 19 days after intracerebral inoculation. A lymphocyte has penetrated into the cytoplasm of a nerve-cell of the *Cornu Ammonis*. N = nucleolus; L = lymphocytes.

FIG. 4. Staining, Mann. ½ 1/12 oil immersion, oc. 4.
Rabbit 80A dead 28 days after intracerebral infection. Mid-brain, oxyphilic degeneration of the nuclear chromatin forming a homogeneous 'block'. C = degenerated nucleus; N = nucleolus; S = satellite cell.

FIG. 5. Staining, Mann. 1/12 oil immersion, oc. 1.
Macacus rhesus No. 1. Spinal ganglion of the lumbar region. Corpuscles of type Joest-Degen in the nucleus of the ganglion cells. C = intranuclear corpuscle surrounded by a halo; N = nucleolus.

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ILLUSTRATIONS

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

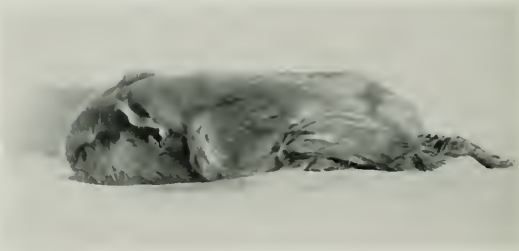

Fig. 7

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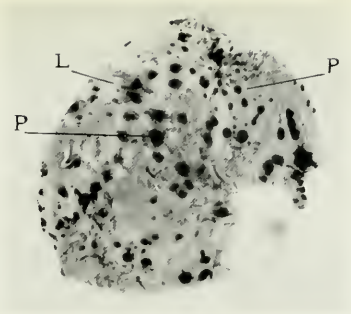

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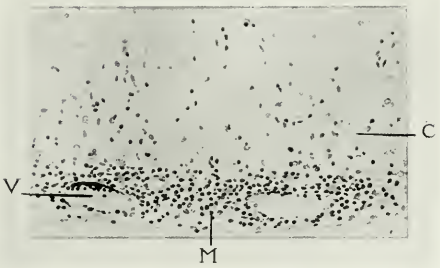

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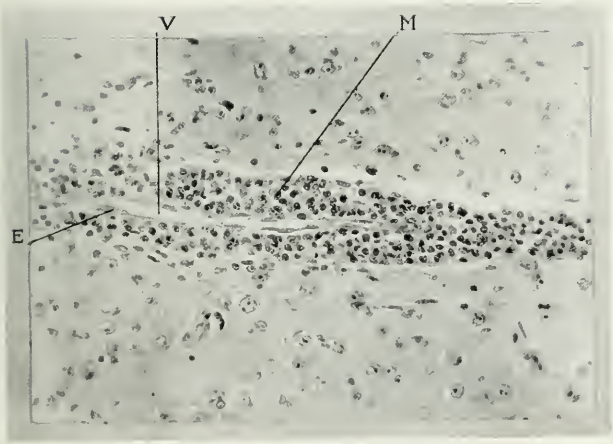

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Fig. 12

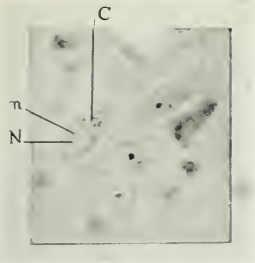
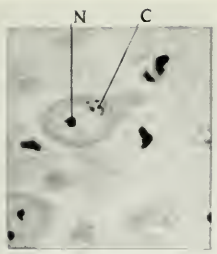


Fig. 13



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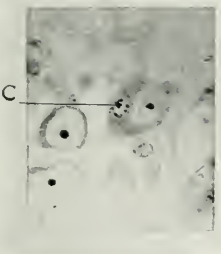

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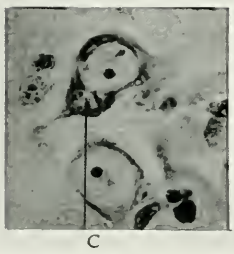

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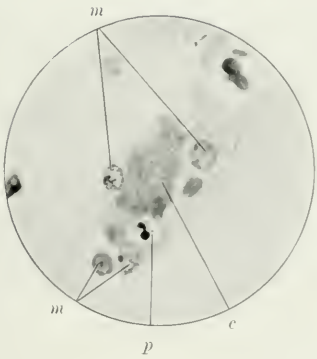

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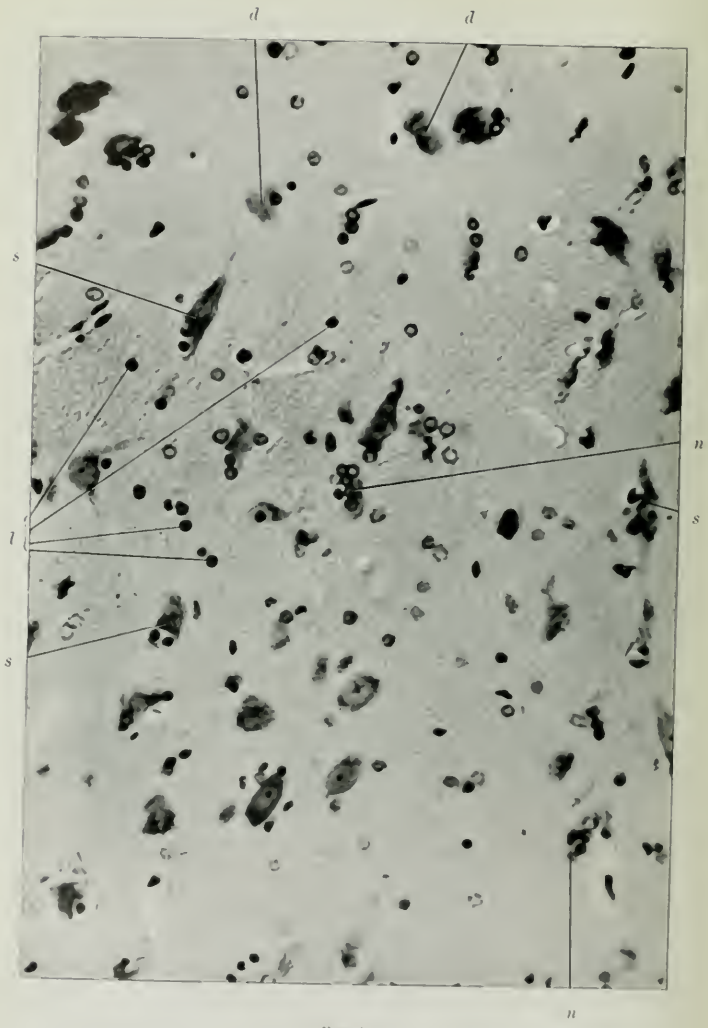

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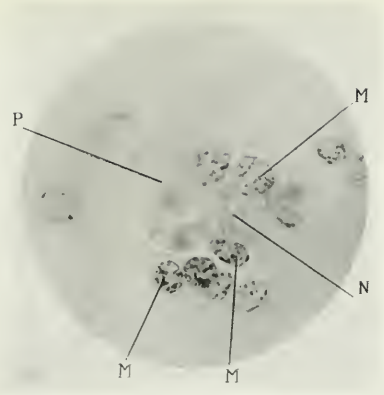

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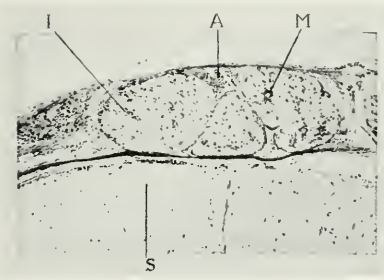

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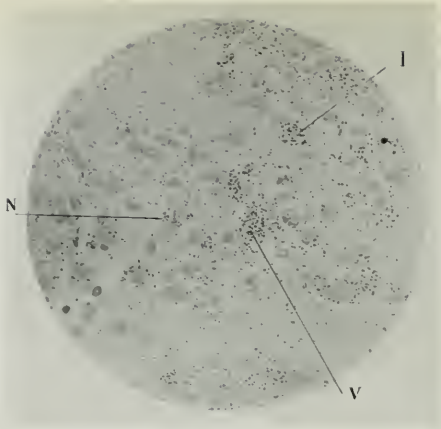

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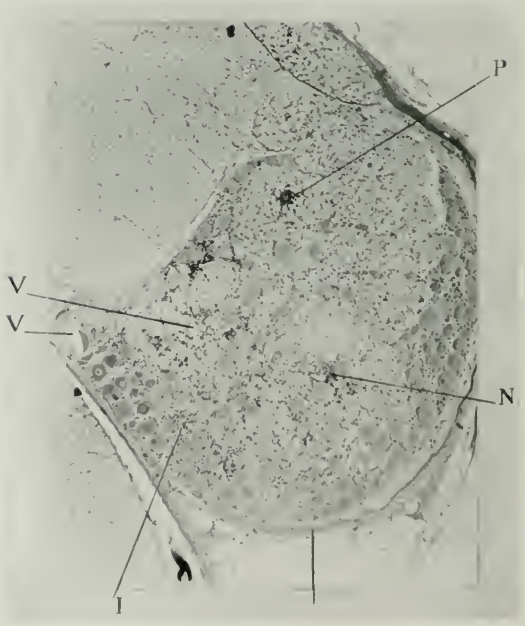

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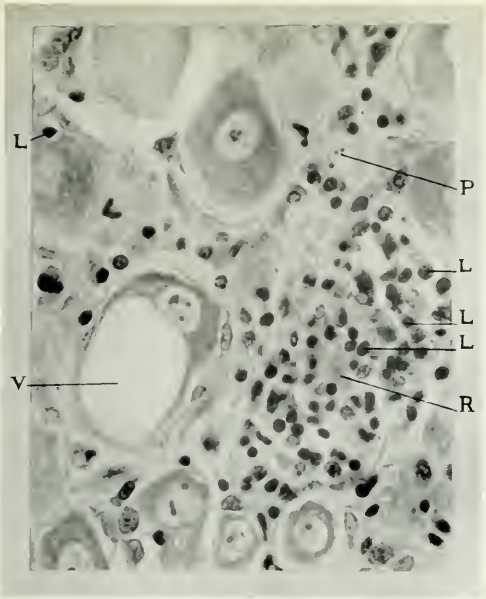

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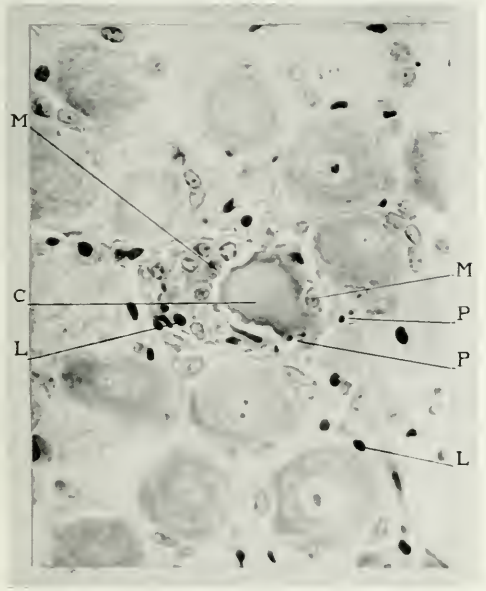

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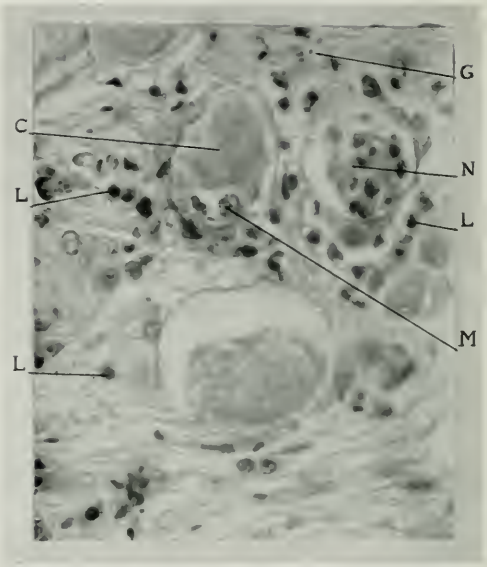

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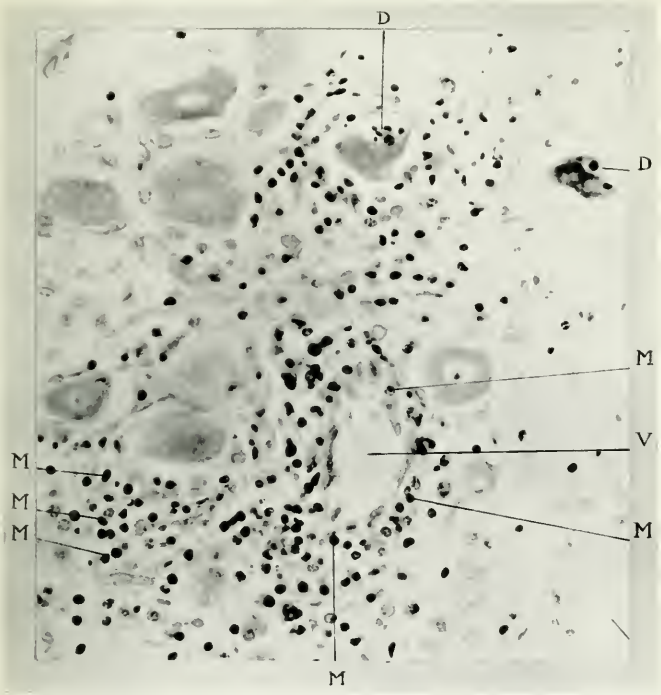

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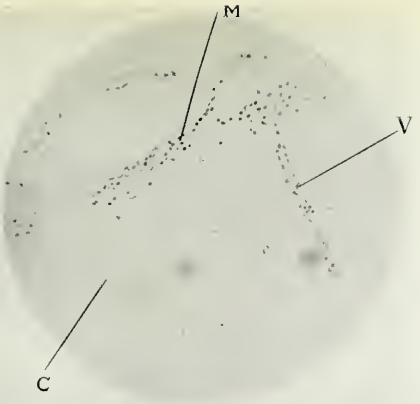

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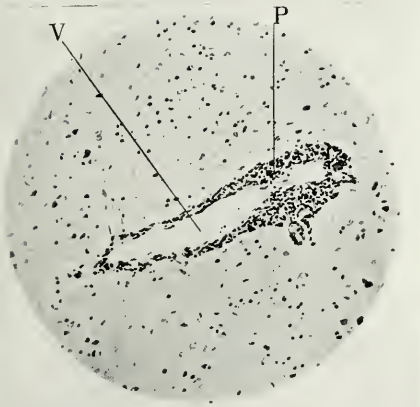

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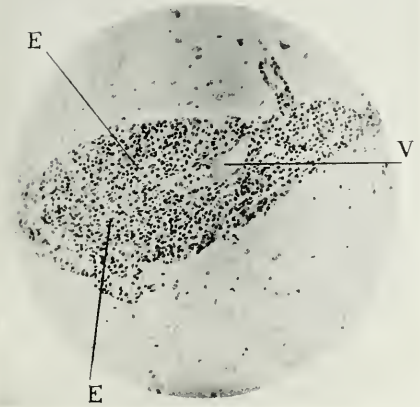

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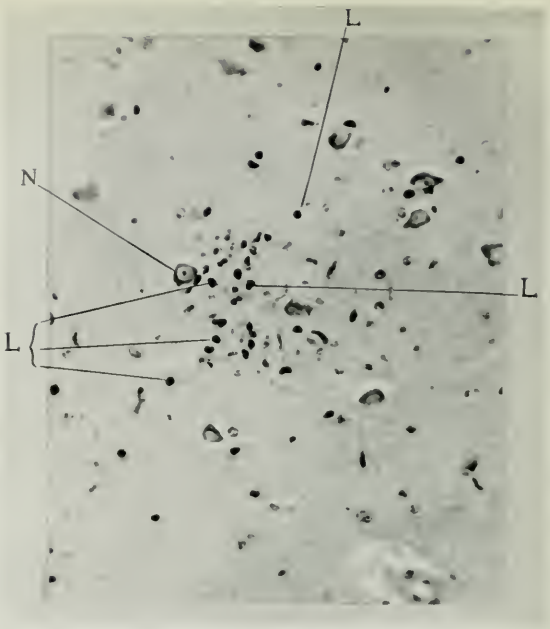

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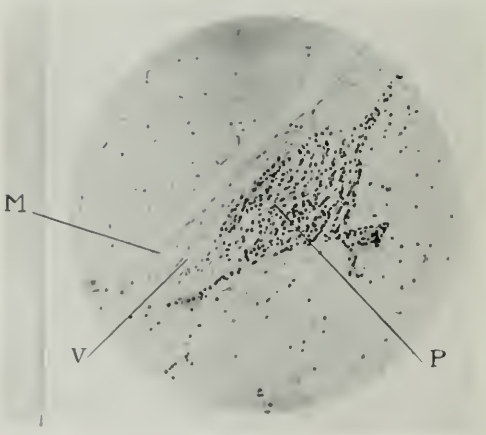

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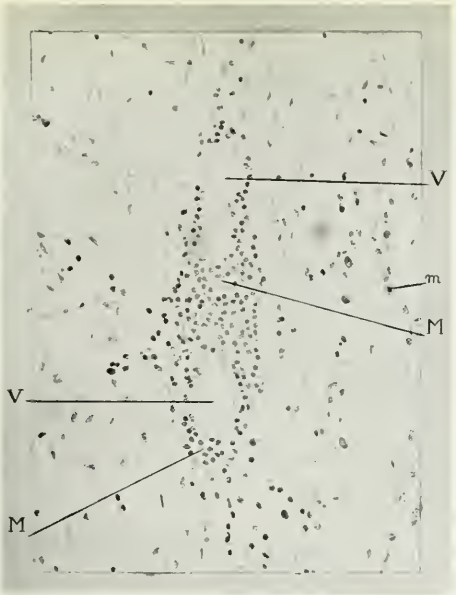

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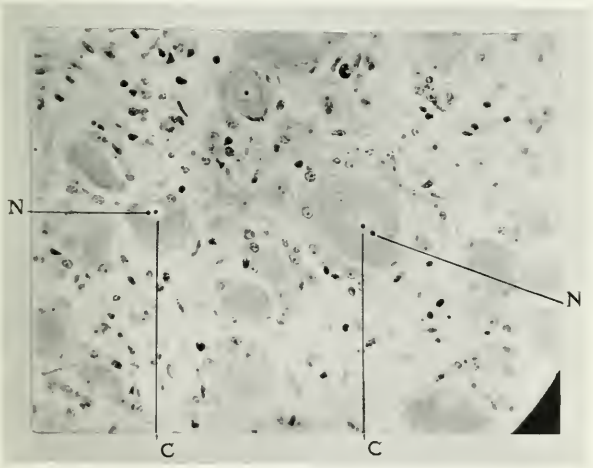

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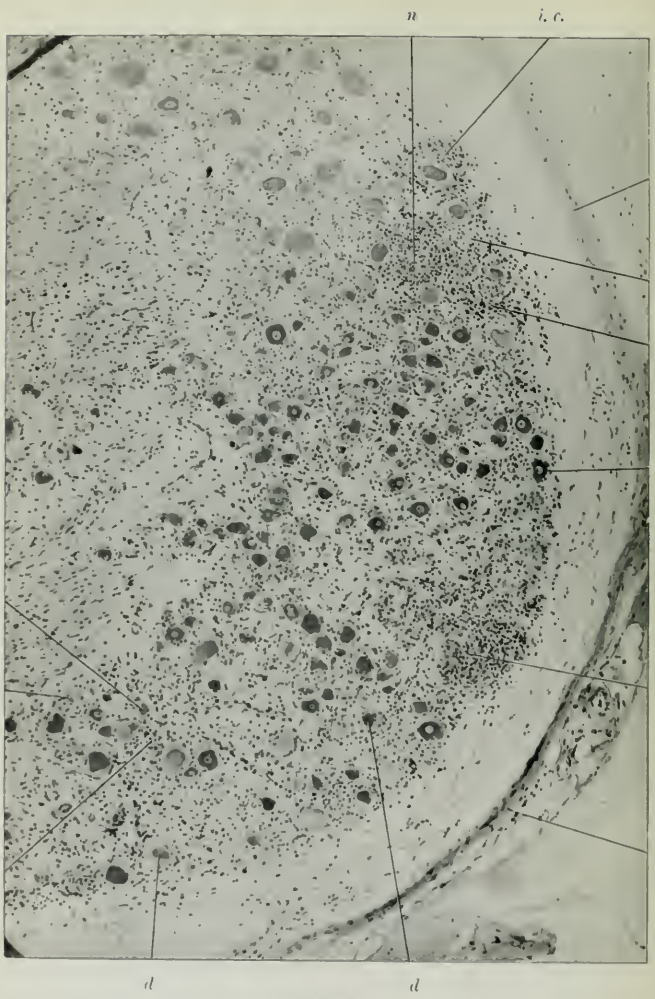

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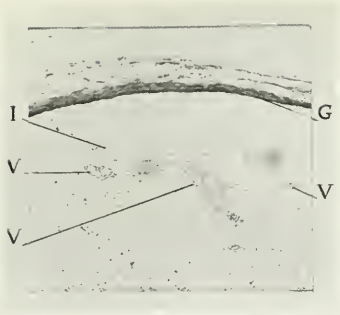

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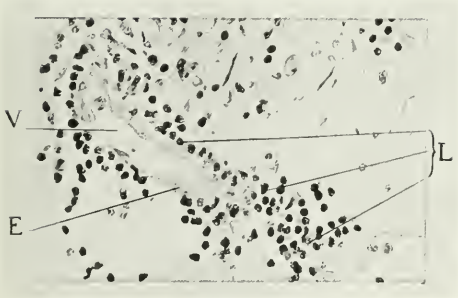

Fig. 38

Fig. 39

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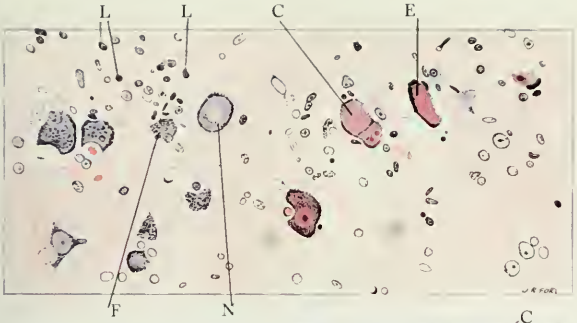

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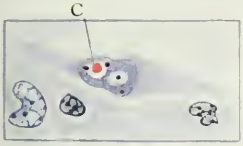

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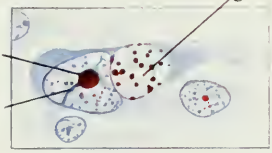

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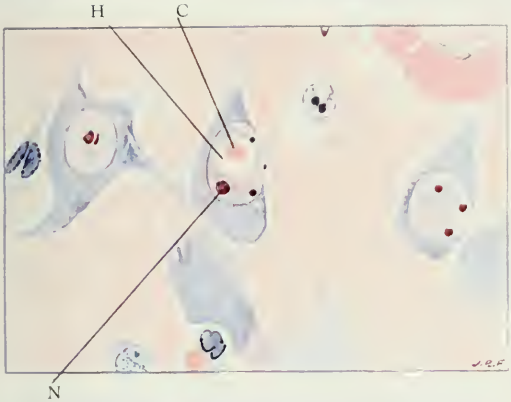

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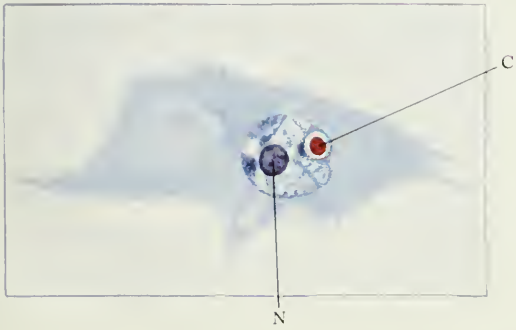

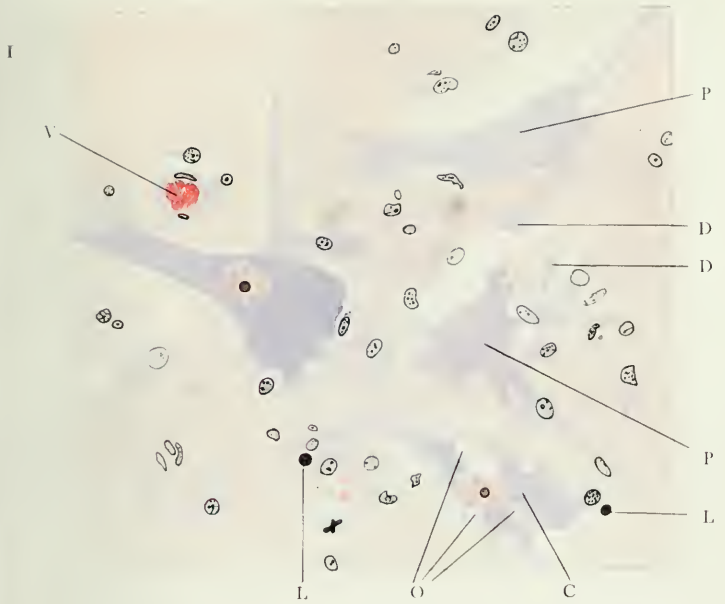
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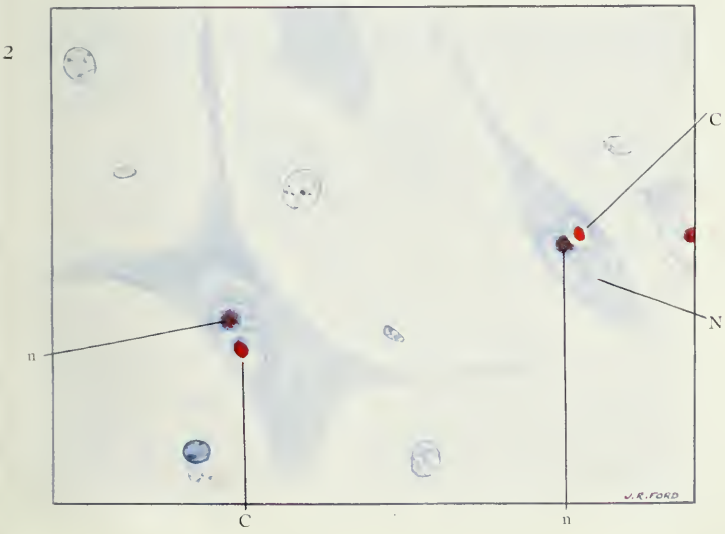
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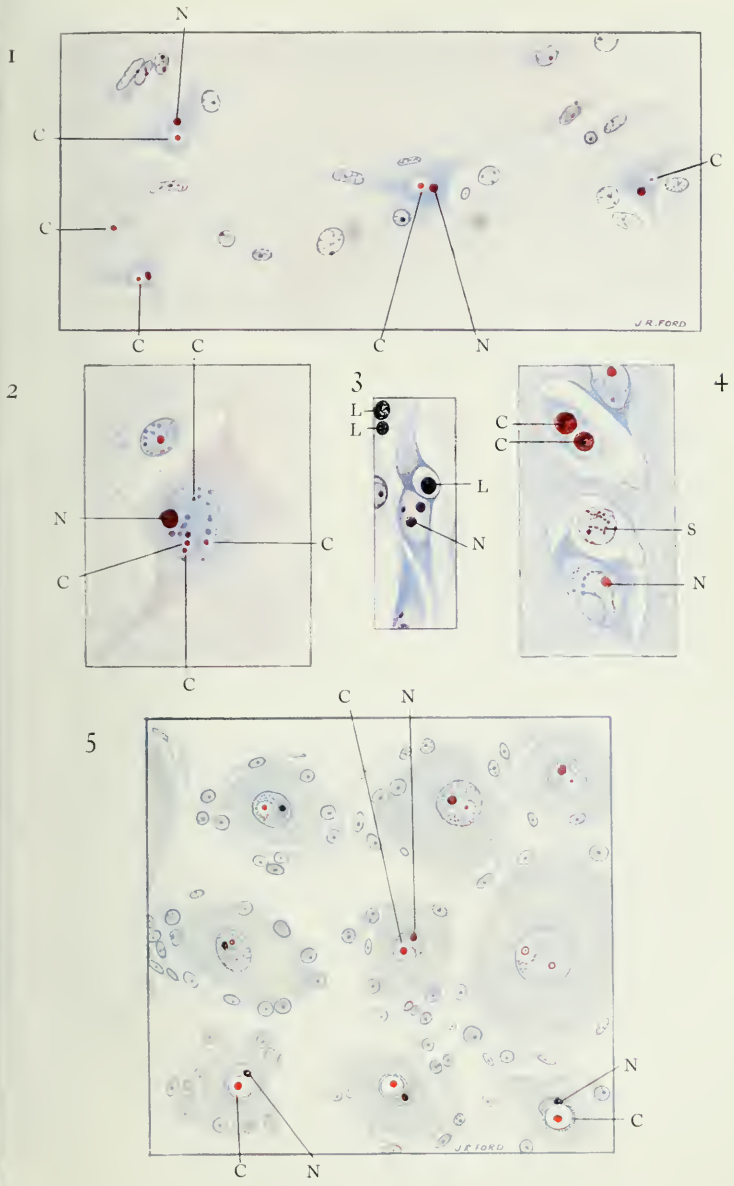
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LIST OF PUBLICATIONS

September, 1928.

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In addition, numerous memoirs upon work aided by the Medical Research Council have appeared in Scientific Journals: particulars of these may be seen in the Annual Reports.

ANNUAL REPORTS

Medical Research Committee, Nos. 1–5, 1914–15 to 1918–19.

Medical Research Council, 1919–20 to 1926–7.

(Price of each report from 1920–1 to 1925–6, 3*s.* 6*d.*, post free 3*s.* 8*d.*; 1926–7 [Cmd. 3013], 3*s.*, post free 3*s.* 2*d.*)

SPECIAL REPORTS, &c.

Alcohol:

No. 31. Alcohol—Its Absorption into and Disappearance from the Blood under different conditions. By E. Mellanby. [1919.] *Out of print.*

No. 34. The Influence of Alcohol on Manual Work and Neuro-muscular Co-ordination. By H. M. Vernon. [1919.] Price 2*s.*, post free 2*s.* 1*d.*

No. 56. The Effects of Alcohol and some other Drugs during Normal and Fatigued Conditions. By W. McDougall and May Smith. [1920.] Price 1*s.*, post free 1*s.* 1*d.*

(Book). Alcohol: its Action on the Human Organism. Second Edition. [1924.] Price 1*s.* paper covers, 1*s.* 6*d.* cloth bound.

Anaerobic Bacteria: *see* Wound Infections.

Animals, Diseases of:

No. 121. Borna Disease and Enzootic Encephalo-Myelitis of Sheep and Cattle. By S. Nicolau and I. A. Galloway. [1928.]

Bacteriology (Miscellaneous):

No. 35. The Reaction of Culture Media, by S. R. Douglas, J. W. H. Eyre, P. P. Laidlaw, and C. G. L. Wolf. Second Edition, revised by P. P. Laidlaw, [1927.] Price 6*d.*, post free 7*d.*

No. 49. On the Destruction of Bacteria in Milk by Electricity. By J. M. Beattie and F. C. Lewis. [1920.] Price 9*d.*, post free 10*d.*

No. 51. The Laboratory Diagnosis of Acute Intestinal Infections, including the Principles and Practice of the Agglutination Tests. By the Committee upon Pathological Methods. [1920.] Price 4*s.* 6*d.*, post free 4*s.* 8*d.*

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See also Shock, Surgical.

* For overseas agencies, see p. viii.

Cancer :

No. 99. An Investigation into the Statistics of Cancer in Different Trades and Professions. By Matthew Young and W. T. Russell. [1926.] Price 1*s.* 6*d.*, post free 1*s.* 7*d.*

See also RADIUM.

Cerebro-spinal Fever :

No. 2. Report of the Special Advisory Committee upon Bacteriological Studies of Cerebro-spinal Fever during the Epidemic of 1915. [1916.] *Out of print.*

No. 3. Bacteriological Studies in the Pathology and Preventive Control of Cerebro-spinal Fever among the Forces during 1915 and 1916. By M. H. Gordon, Martin Flack, P. W. Bassett-Smith, and T. G. M. Hine and W. J. Tulloch. [1917.] *Out of print.*

No. 17. (I.) A Report upon the Seasonal Outbreak of Cerebro-spinal Fever in the Navy at Portsmouth, 1916–17. By Paul Fildes and S. L. Baker. (II.) The Treatment of Cerebro-spinal Meningitis by Antimeningococcus Serum at the Royal Naval Hospital, Haslar, 1915–16–17. By G. P. Adshead. [1918.] Price 2*s.* 6*d.*, post free 2*s.* 8½*d.*

No. 50. Cerebro-spinal Fever. Studies in the Bacteriology, Preventive Control, and Specific Treatment of Cerebro-spinal Fever among the Military Forces, 1915–19. By M. H. Gordon and others. [1920.] Price 4*s.*, post free 4*s.* 3*d.*

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Child Life (ANTENATAL and POSTNATAL INVESTIGATIONS) :

No. 10. The Mortalities of Birth, Infancy, and Childhood. By A. K. Chalmers, W. A. Brend, L. Findlay, and J. Brownlee. [1917.] Price 1*s.* 6*d.*, post free 1*s.* 7½*d.*

No. 74. The Relation between Home Conditions and the Intelligence of School Children. By L. Isserlis. [1923.] Price 1*s.*, post free 1*s.* 1*d.*

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No. 101. Poverty, Nutrition, and Growth : Studies of Child Life in Cities and Rural Districts of Scotland. [1926.] By D. Noël Paton, Leonard Findlay, and others. Price 10*s.*

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No. 70. The Structure of Teeth in relation to Dental Disease. By J. Howard Mummery. [1922.] Price 2*s.*, post free 2*s.* 1*d.*

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No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] Price 2*s.*, post free 2*s.* 1½*d.*

(Book). Diphtheria ; its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1923.] Price 12*s.* 6*d.*, post free 13*s.* 3*d.*

See also EPIDEMIOLOGY (No. 75).

Dysentery :

Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean : —

No. 4. I. Amoebic Dysentery and the Protozoological Investigation of Cases and Carriers. By Clifford Dobell. [1917.] *Out of print.*

No. 5. II. Report upon 878 Cases of Bacillary Enteritis. By L. Rajchman and G. T. Western. [1917.] *Out of print.*

No. 6. III. Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915–16. By Paul Fildes and others. IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] Price 4*s.* 6*d.*, post free 4*s.* 7½*d.*

No. 7. V. Report upon 2,360 Enteritis 'Convalescents' received at Liverpool from various Expeditionary Forces. By E. Glynn and others. [1918.] Price 6*s.*, post free 6*s.* 2*d.*

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals : with special reference to the Incidence and Treatment of Amoebic Dysentery Carriers. By Clifford Dobell, H. S. Gettings, Margaret W. Jepps, and J. B. Stephens. [1918.] Price 1*s.* 3*d.*, post free 1*s.* 4*d.*

No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. By W. Fletcher and Doris L. Mackinnon. [1919.] Price 9*d.*, post free 10*d.*

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See also Food Poisoning.

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No. 108. The Sheffield Outbreak of Encephalitis in 1924. [1926.] Price 1*s.* 9*d.*, post free 1*s.* 10½*d.*

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Haemoglobin : *see* BLOOD.

Heart Disease :

No. 8. Report upon Soldiers returned as Cases of 'Disordered Action of the Heart' (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] Price 1*s.*, post free 1*s.* 1*d.*

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(The Annual Reports of the Industrial Fatigue Research Board, and special reports on particular subjects, are published for the Council in separate series. The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 15 York Buildings, Adelphi, W.C. 2.)

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Miners' Dietaries : *see* NUTRITION.

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See also JAUNDICE (No. 113).

Nephritis :

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Pituitary Extract : *see* STANDARDS.

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Pneumothorax, Artificial : *see* TUBERCULOSIS.

Print, Legibility of : *see* VISION.

Protozoan Infections :

No. 59. A Report on the Occurrence of Intestinal Protozoa in the inhabitants of Britain. By Clifford Dobell. [1921.] Price 2*s.*, post free 2*s.* 1½*d.*

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No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] Price 1*s.*, post free 1*s.* 1*d.*

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Rheumatism : *see* CHILD LIFE (No. 114).

Rickets :

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*

No. 61. Experimental Rickets. By E. Mellanby. [1921.] Price 4*s.*, post free 4*s.* 2*d.*

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No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with other factors of Diet and Environment in producing Rickets. By E. Mellanby. [1925.] Price 3*s.*, post free 3*s.* 8*d.*

Salvarsan : *see* Venereal Diseases *and* Streptococcal Infections.

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Conditions :—

No. 25. Wound-Shock and Haemorrhage. [1919.] Price 4*s.*, post free 4*s.* 5½*d.*

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No. 98. Studies of the Viruses of Vaccinia and Variola. By M. H. Gordon. [1925.] Price 3*s.* 6*d.*, post free 3*s.* 8½*d.*

No. 106. Small-pox and Climate in India : Forecasting of Epidemics. By Sir Leonard Rogers. [1926.] Price 2*s.*, post free 2*s.* 1½*d.*

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See also Venereal Diseases (No. 44).

Statistics (Miscellaneous).

No. 16. A Report on the Causes of Wastage of Labour in Munition Factories. By Major Greenwood. [1918.] Price 1*s.* 6*d.*, post free 1*s.* 7*d.*

No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] Price 3*s.*, post free 3*s.* 1½*d.*

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Tuberculosis :

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] Price 3*d.*, post free 3½*d.*

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.] Price 1*s.* 3*d.*, post free 1*s.* 4½*d.*

No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Munition Workers. By Major Greenwood and A. E. Tebb. [1919.] Price 1*s.* 6*d.*, post free 1*s.* 7*d.*

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No. 21. The Diagnostic Value of the Wassermann Test. By the Committee upon Pathological Methods. [1918.] Price 1*s.*, post free 1*s.* 1*d.*

No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of Syphilis or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] Price 2*s.*, post free 2*s.* 1*d.*

No. 41. (I.) An Investigation into the Ultimate Results of the Treatment of Syphilis with Arsenical Compounds. By Paul Fildes and R. J. G. Parnell. (II.) A Clinical Study of the Toxic Reactions which follow the Intravenous Administration of '914'. By R. J. G. Parnell and Paul Fildes. [1919.] Price 2*s.*, post free 2*s.* 1*d.*

No. 44. Reports of the Special Committee upon the Manufacture, Biological Testing, and Clinical Administration of Salvarsan and of its Substitutes. I. [1919.] Price 1*s.*, post free 1*s.* 1*d.*

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No. 47. The Accuracy of Wassermann Tests, applied before and after death, estimated by Necropsies. I. The Wassermann Test applied before death. By H. M. Turnbull. [1920.] Price 2*s.* 6*d.*, post free 2*s.* 7½*d.*

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